

	Gly205Asn + Thr207Gln
	Ser210Glu + Leu211Pro
	Leu 94Gln + Ser210Glu
	Ala209Asp + Leu211Pro
5	Asn 60Glu + Leu211His
	Ala 96Asn + Thr214Gly
	Asn 60Glu + Ala194Gly
	Ser103Asp + Ala181His
	Gly125Glu + Ala209Asn
10	Ser158Asp + Asn198Ser
	Gly155Ser + Asn198Ser
	Ser104Asp + Gly152Asn
	Ser182Asp + Leu211Cys
	Val 93Ser + Gly125Glu
15	Asn198Glu + Leu211Ser
	Gly100Ser + Ser103Asp
	Ala181Gln + Gly213Gln
	Thr 64Gly + Ser126Asp
	Ile159Cys + Gln200Asp
20	Thr207Glu + Leu211Asn
	Ser104Glu + Pro204Gly
	Ser130Asp + Pro204Asn
	Pro129Gln + Gln185Asn
	Asn 60Glu + Gly157Ser
25	Gly125Pro + Val197Met
	Asn 60Glu + Ile105Cys
	Pro127Gln + Val197Thr
	Ser101Asp + Leu211Ala
	Gln 57Ser + Ser 99Glu
30	Phe183Pro + Asn198Glu
	Asn198Glu + Thr202Pro
	Val197Gln + Leu211His
	Gly 61Ser + Gln200Ser
	Gly 61Asp + Asn198Gln
35	Gly 61Glu + Phe183Ser
	Thr207Asn + Asn212Glu
	Tyr203Thr + Tyr208Gly
	Asn198Ser + Leu211Cys
	Asn153Asp + Leu211Ser
40	Gln 57Asn + Gly213Pro
	Thr 64Ser + Ser210Asp
	Pro204Asp + Leu211Ile
	Gln 57Asp + Gly 61Pro
	Gly155Gln + Ser210Asp
45	Ala156Gly + Thr207Asn
	Gly 61Gln + Ala181Thr
	Ile159Cys + Asn198Gln
	Pro129Gly + Leu211Glu
	Asn198Glu + Thr207Ser
50	Gly 98Ser + Ser128Asp
	Ala131Asn + Val193Ser
	Gln185Ser + Gln200Asn
	Ser210Asp + Leu211His
	Gly125Glu + Ala156Gln

	Ser 97Asp + Gly125Gln
	Asn 60Gln + Gly155Pro
	Ser126Glu + Thr207Ser
	Phe183Met + Thr207Pro
5	Ser154Glu + Phe183Ile
	Val 93Gln + Ser210Asp
	Val 93Gln + Tyr208His
	Asn 60Ser + Asn198Glu
	Gly152Gln + Gln200Asp
10	Pro127Asn + Gly152Asn
	Gly155Gln + Thr214Asn
	Ala 96Ser + Tyr208His
	Tyr161Thr + Ser210Asp
	Gly100Pro + Pro204Gln
15	Gln 57Asp + Ala194Asn
	Asn 60Gln + Thr202Asn
	Gly157Pro + Gly213Asn
	Val 93Thr + Leu211Ala
	Ser103Glu + Leu211Thr
20	Ser101Asp + Leu124Ile
	Leu 94Glu + Pro204Gln
	Gln200Glu + Ala209His
	Ser101Glu + Gly152Gln
	Asn198Asp + Gly205Pro
25	Gly 61Ser + Gln200Asn
	Ser158Asp + Val197Asn
	Gly 61Glu + Phe183Ile
	Asp 58Glu + Tyr203Ser
	Gly213Ser + Thr214Gly
30	Gln 57Asp + Asn198Gln
	Tyr102Leu + Ser158Glu
	Ala 96Gln + Leu211Asn
	Val 93Asp + Thr202Asn
	Leu 94Ser + Asn198Asp
35	Gly196Ser + Asn212Asp
	Gly155Asn + Gly157Asp
	Val 93Asn + Ser130Asp
	Leu 94Ile + Ser210Asp
	Val197Ala + Ser210Asp
40	Ser104Asp + Gly205Pro
	Asn153Asp + Ala181Gly
	Gln200Asn + Leu211Glu
	Leu 94Asn + Ser210Glu
	Gln185Asp + Val197Gln
45	Tyr102Ser + Thr207Asp
	Gly 61Glu + Gly 95Asn
	Gly 61Asp + Pro129Gln
	Ser 99Asp + Thr207Ser
	Ser126Glu + Gly152Gln
50	Val197His + Ser206Glu
	Gly 61Asn + Gly155Glu
	Gly155Asn + Asn198Glu
	Ala209Asn + Ser210Glu
	Ser128Asp + Thr207Gly

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	Ala209Thr + Ser210Asp
	Gln185Asn + Gly213Pro
	Ser126Glu + Leu211Gly
5	Ser 97Asp + Gly203Ser
	Leu 94Thr + Leu124Thr
	Asn 60Gln + Leu211Gly
	Pro129Asp + Gly155Pro
	Asp 58Glu + Gly157Ser
	Thr202Pro + Thr207Gln
10	Ala156Ser + Tyr208Asp
	Gly 98Glu + Tyr161Gln
	Ile105Cys + Gln200Asn
	Gln200Glu + Pro204Gly
	Gly 98Pro + Ala131Asn
15	Gly 59Asp + Thr207Gln
	Tyr102Gln + Ala209Gln
	Gly 98Glu + Ile105His
	Ser101Asp + Pro129Gln
	Pro127Asp + Gly213Asn
20	Ser104Glu + Asn198Gln
	Gln 57Ser + Gln200Asn
	Val197Cys + Gln200Ser
	Val 93Ala + Ala156Glu
	Gln200Asn + Gly205Glu
25	Leu 94Ala + Thr207Asn
	Ala131Thr + Gln200Ser
	Leu 94Glu + Tyr161Thr
	Gln200Asn + Ser210Asp
	Gln 57Asp + Thr207Asn
30	Ser 99Glu + Ala156Pro
	Leu124Ile + Asn198Ser
	Tyr161Gly + Thr207Gln
	Ile105Cys + Thr214Asp
	Ile159His + Ser206Asp
35	Gly 63Gln + Gly123Asn
	Asn198Asp + Gly205Ser
	Ser104Glu + Leu124Gln
	Ala209Pro + Ser210Glu
	Tyr161Val + Asn198Asp
40	Pro129Ser + Gln200Asp
	Ser 97Asp + Tyr203Ser
	Ser103Asp + Phe183Thr
	Tyr161Gly + Val197Ala
	Asn198Gln + Pro204Glu
45	Leu211Gly + Asn212Glu
	Ser206Glu + Leu211Thr
	Gly152Pro + Val197Ser
	Ile105Ala + Ala156Gln
	Gly 63Asn + Tyr102Pro
50	Ser182Glu + Val197Asn
	Thr 64Glu + Ala 96Gln
	Asn 60Gln + Ser206Glu
	Asn 60Gln + Ser128Asp
	Ser101Asp + Ile159Pro

	Gly100Ser + Thr207Ser
	Gly 63Asn + Gly205Ser
	Ala181Glu + Thr207Asn
5	Gly125Ser + Tyr161Ser
	Gln200Glu + Leu211Ala
	Ser101Asp + Tyr102Leu
	Ser126Glu + Thr207Pro
	Gly 59Asp + Tyr102Thr
10	Gly100Gln + Tyr102Met
	Gly 59Ser + Asn198Asp
	Asn 60Ser + Leu 94Cys
	Pro127Glu + Gly205Pro
	Gln 57Glu + Ala156Asn
15	Ala131Asn + Thr207Asp
	Ala131Asp + Thr202Ser
	Leu124His + Ala131Asn
	Gly152Glu + Leu211His
	Ser101Glu + Ala181Thr
20	Asp 58Glu + Gly205Ser
	Gly 61Asn + Gln200Asp
	Pro129Gly + Val197Asp
	Thr 64Gln + Asn198Ser
	Val 93Ala + Ser154Glu
	Ser130Glu + Tyr161Val
25	Phe183Asp + Leu211Thr
	Ser206Glu + Asn212Gln
	Gly 63Ser + Ala131Glu
	Pro127Asn + Gln200Asp
30	Ser130Asp + Phe183Thr
	Ala181Thr + Pro195Ser
	Gly 95Ser + Ala156Glu
	Tyr161Gln + Tyr208Ile
	Gln 57Asp + Leu211Cys
35	Asn153Gln + Gly213Asn
	Val 93Thr + Leu124Ser
	Ser101Glu + Asn212Gln
	Gly157Ser + Asn198Glu
	Gly152Ser + Ser182Glu
	Tyr161Gln + Gly213Ser
40	Pro195Gly + Gln200Asn
	Asn 60Asp + Leu211Pro
	Pro195Ser + Tyr208Glu
	Gln200Asp + Leu211Asn
	Leu124Val + Thr207Pro
45	Thr 64Pro + Ser126Glu
	Thr 64Ser + Tyr203Val
	Ser 97Glu + Asn198Ser
	Gly152Gln + Leu211Pro
	Asn198Asp + Leu211Asn
50	Val 93Glu + Gly125Gln
	Gly 98Asn + Gln200Asp
	Gln200Asn + Thr207Asp
	Asn198Ser + Gln200Glu
	Tyr102Leu + Asn198Gln

	Leu124Cys + Asn153Ser
	Asn198Asp + Leu211Pro
	Ser126Glu + Tyr208Met
5	Ala 96Asn + Gly100Ser
	Ser 99Glu + Leu211Cys
	Ser 97Asp + Leu211Ala
	Ser182Glu + Leu211His
	Ile159Cys + Leu211Glu
	Gly152Asn + Ser210Asp
10	Val197Ala + Gln200Glu
	Asn 60Ser + Ser158Glu
	Ser104Glu + Tyr208Asn
	Pro204Asp + Leu211Gly
	Asp 58Glu + Val197Gly
15	Ser182Glu + Tyr208Ser
	Ser103Asp + Ala194Ser
	Ala156Gln + Thr207Asp
	Asn198Ser + Thr207Ser
	Gln 57Asp + Thr207Ser
20	Gly152Glu + Thr207Gly
	Ala131Ser + Ile159Asn
	Pro127Asn + Gly152Glu
	Gln200Asn + Gly213Glu
	Leu124Asp + Leu211Ala
25	Gly 98Glu + Gly125Asn
	Ile159Gly + Thr207Asn
	Pro127Gln + Asn153Asp
	Ser103Glu + Phe183Tyr
	Ser160Glu + Pro204Ser
30	Gly157Ser + Tyr161His
	Ala156Pro + Ser158Glu
	Gly100Glu + Pro127Asn
	Gln 57Asn + Asn 60Glu
	Gly125Ser + Ser210Glu
35	Gly125Pro + Ser128Asp
	Gly125Glu + Val197Gly
	Val193Met + Asn198Ser
	Ser160Glu + Gly213Asn
	Ser206Glu + Ala209His
40	Ser 97Glu + Gly213Ser
	Gly100Asp + Leu211Gly
	Pro129Gln + Thr207Gly
	Gly 95Gln + Ser210Asp
	Gln185Ser + Pro204Glu
45	Pro129Glu + Ala156Ser
	Ser126Asp + Asn212Ser
	Gly 59Ser + Ile159Ala
	Ala 96Asn + Ser 99Glu
	Ser104Asp + Ile159Val
50	Gly 63Asn + Ser210Glu
	Phe183Pro + Ser210Glu
	Ala 96Asp + Tyr208Ile
	Gly 98Asp + Thr207Gln
	Val 93Gln + Gly125Glu

	Ile159Glu + Thr207Gln
	Leu 94Ile + Leu211Val
	Gly157Asp + Leu211His
5	Gly 61Asp + Leu211Cys
	Gln 57Asn + Gly157Asn
	Gly100Glu + Val197Ser
	Gln200Glu + Pro204Asn
	Ala131Gly + Leu211His
	Ser206Asp + Leu211Asn
10	Asn153Asp + Ile159Leu
	Gly152Ser + Asn198Glu
	Leu 94Met + Ser130Glu
	Gln 57Glu + Asn198Gln
	Ser182Glu + Leu211Thr
15	Val199Ala + Ser210Glu
	Thr207Gly + Ser210Glu
	Thr 64Gly + Gly 98Asp
	Gly 61Gln + Ser 99Asp
	Gly155Glu + Leu211Ser
20	Leu124Ser + Thr207Asp
	Val 93Ser + Asn198Gln
	Ser 99Asp + Gly125Asn
	Gln 57Asn + Asn198Glu
	Ser 99Glu + Ile159Met

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## Multi-loop Triple Mutation Variants

	Gln 57Ser + Leu 94Gly + Gln200Glu
	Asn 60Ser + Val 93Gln + Gly213Asp
30	Tyr102Cys + Asn198Gln + Thr207Gly
	Leu124Ile + Ser154Glu + Asn198Gln
	Leu 94Gly + Ala209Gly + Ser210Glu
	Gly155Asp + Ala209Gln + Asn212Gln
	Pro129Asn + Gly157Asn + Thr207Ser
35	Asn198Gln + Gln200Asp + Ala209Pro
	Val 93Ala + Pro127Asp + Thr202Ser
	Tyr161Asn + Thr207Asp + Leu211Met
	Gly 61Ser + Pro127Asn + Thr207Pro
	Gly 61Pro + Leu124Asp + Pro204Asn
40	Gln 57Glu + Gln185Ser + Asn198Ser
	Gly 59Asn + Asn 60Glu + Gln200Ser
	Gly 63Gln + Gln200Asp + Leu211Gly
	Tyr203Ser + Thr207Gly + Gly213Glu
	Gly 63Asn + Gln185Asp + Ala209Asn
45	Pro129Asn + Ser210Glu + Leu211Asn
	Tyr102Ala + Gly125Glu + Leu211Gly
	Gln 57Asn + Gly155Gln + Leu211His
	Ile105Pro + Gly125Ser + Thr207Gly
	Asn198Ser + Thr207Asn + Ser210Asp
50	Gly 59Gln + Gly 95Ser + Ala156Glu
	Tyr102Cys + Ser210Glu + Leu211Asn
	Gly205Asn + Thr207Gln + Ser210Glu

	Leu 94Gln + Ala209Asp + Leu211Pro
	Asn 60Glu + Leu211His + Thr214Gly
	Asn 60Glu + Ala181His + Ala194Gly
	Ser104Asp + Gly155Ser + Asn198Ser
5	Gly152Asn + Ser182Asp + Leu211Cys
	Gly100Ser + Ser103Asp + Leu211Ser
	Thr 64Gly + Ser126Asp + Ile159Cys
	Ser130Asp + Ala181Asn + Pro204Gly
	Pro129Gln + Gly157Ser + Gln185Asn
10	Asn 60Glu + Gly125Pro + Val197Met
	Ile105Cys + Pro127Gln + Val197Thr
	Gln 57Ser + Ser 99Glu + Phe183Pro
	Gly 61Ser + Val197Gln + Leu211His
	Gly 61Asp + Asn198Gln + Gln200Ser
15	Tyr203Thr + Thr207Asn + Tyr208Gly
	Asn153Asp + Asn198Ser + Leu211Ser
	Gln 57Asn + Ser210Asp + Gly213Pro
	Thr 64Ser + Pro204Asp + Leu211Ile
	Ser101Asp + Ala156Gly + Thr207Asn
20	Ile159Cys + Ala181Thr + Asn198Gln
	Gly 98Ser + Asn198Glu + Thr207Ser
	Ser128Asp + Ala131Asn + Val193Ser
	Gln185Ser + Gln200Asn + Leu211His
	Ser126Glu + Phe183Met + Thr207Ser
25	Ser154Glu + Phe183Ile + Thr207Pro
	Val 93Gln + Tyr208His + Ser210Asp
	Val 93His + Pro127Asn + Gly152Gln
	Gly155Gln + Gly213Glu + Thr214Asn
	Gly100Pro + Tyr161Thr + Ser210Asp
30	Gln 57Asp + Ala194Asn + Pro204Gln
	Gly157Pro + Thr202Asn + Gly213Asn
	Thr 64Gln + Val 93Thr + Leu211Ala
	Gly 61Ser + Gln200Asn + Gly205Pro
	Ser158Asp + Phe183Ile + Val197Asn
35	Asn198Asp + Gly213Ser + Thr214Gly
	Gly152Ser + Gly155Ser + Ser206Asp
	Gly152Gln + Gln185Ser + Val197Gln
	Leu 94Ile + Val197Ala + Ser210Asp
	Ala181Gly + Gln200Asn + Leu211Glu
40	Leu 94Asn + Val197Gln + Ser210Glu
	Gly 61Asp + Pro129Gln + Thr207Ser
	Gln 57Asn + Gly 61Asn + Gly155Glu
	Gly 61Pro + Ser128Asp + Ala209Thr
	Thr207Gly + Ala209Thr + Ser210Asp
45	Ser128Asp + Gln185Asn + Gly213Pro
	Leu 94Thr + Leu124Thr + Gly205Ser
	Asn 60Gln + Gly155Pro + Leu211Gly
	Asp 58Glu + Gly157Ser + Thr202Pro
	Gly 98Glu + Ile105Cys + Gln200Asn
50	Gly 98Pro + Gln200Glu + Pro204Gly
	Gly 59Asp + Ala131Asn + Thr207Gln
	Gly 98Glu + Tyr102Gln + Ala209Gln
	Ser101Asp + Ile105His + Pro129Gln
	Pro127Asp + Asn198Gln + Gly213Asn

	Ile159Gly + Val197Gly + Asn198Gln
	Gln 57Ser + Gly155Glu + Gln200Asn
	Val197Cys + Asn198Glu + Gln200Ser
	Leu 94Ala + Gln200Asn + Gly205Glu
5	Ala131Thr + Gln200Ser + Thr207Asn
	Leu124Ile + Ala156Pro + Asn198Ser
	Val 93Glu + Tyr161Gly + Thr207Gln
	Gly 63Gln + Gly125Asn + Ala209Glu
	Tyr161Gly + Val197Ala + Asn198Gln
10	Ser158Asp + Asn198Ser + Leu211Gly
	Ile105Ala + Gly152Pro + Val197Ser
	Gly 63Asn + Tyr102Pro + Ala156Gln
	Ala 96Gln + Ser182Glu + Val197Asn
	Asn 60Gln + Ser101Asp + Ile159Pro
15	Gly100Ser + Gly205Ser + Thr207Ser
	Tyr161Ser + Ala181Glu + Thr207Asn
	Gly125Ser + Gln200Glu + Leu211Ala
	Gly 59Asp + Tyr102Thr + Thr207Pro
	Gly 59Ser + Gly100Gln + Tyr102Met
20	Asn 60Ser + Leu 94Cys + Asn198Asp
	Pro127Glu + Ala156Asn + Gly205Pro
	Asp 58Glu + Ala181Thr + Gly205Ser
	Thr 64Gln + Val 93Ala + Asn198Ser
	Phe183Asp + Leu211Thr + Asn212Gln
25	Pro127Asn + Ser130Asp + Phe183Thr
	Tyr161Gln + Tyr208Ile + Leu211Cys
	Leu124Ser + Asn153Gln + Gly213Asn
	Val 93Thr + Ser101Glu + Asn212Gln
	Tyr161Gln + Gln200Asn + Gly213Ser
30	Gln200Asp + Thr207Pro + Leu211Asn
	Thr 64Pro + Leu124Val + Ser126Glu
	Thr 64Ser + Ser 97Glu + Asn198Ser
	Gly152Gln + Ser210Asp + Leu211Pro
	Val 93Pro + Asn198Ser + Gln200Glu
35	Thr 64Gln + Tyr102Leu + Asn153Ser
	Ala 96Asn + Gly100Ser + Tyr208Met
	Ile159Cys + Ser182Glu + Leu211His
	Asn 60Ser + Ser158Glu + Tyr208Asn
	Ser103Asp + Ala194Ser + Tyr208Ser
40	Ala156Gln + Asn198Ser + Thr207Asp
	Ala131Ser + Gly152Glu + Ile159Asn
	Pro127Asn + Gln200Asn + Gly213Glu
	Gly 98Glu + Gly125Asn + Leu211Ala
	Ile159Gly + Thr207Asn + Ser210Glu
45	Ser103Glu + Phe183Tyr + Pro204Ser
	Gly157Ser + Ser160Glu + Tyr161His
	Gln 57Asn + Gly100Glu + Pro127Asn
	Gly125Pro + Val197Gly + Ser210Glu
	Val193Met + Asn198Ser + Gly213Asn
50	Gly 95Gln + Pro129Gln + Thr207Gly
	Gly 59Ser + Ser126Asp + Asn212Ser
	Gly 63Asn + Ile159Val + Ser210Glu
	Phe183Pro + Tyr208Ile + Ser210Glu
	Leu 94Ile + Ile159Glu + Thr207Gln



	Gln 57Asn + Gly157Asn + Leu211Cys
	Ala131Gly + Pro204Asn + Leu211His
	Ile159Leu + Ser206Asp + Leu211Asn
5	Leu 94Met + Gly152Ser + Asn198Glu
	Ser182Glu + Val199Ala + Leu211Thr
	Leu124Ser + Thr207Asp + Leu211Ser
	Val 93Ser + Gly125Asn + Asn198Gln
	Tyr102Pro + Ala156Ser + Phe183His
10	Gly 98Gln + Ser128Glu + Ile159Cys
	Ser104Asp + Ile105Pro + Tyr161Thr
	Tyr102Pro + Ile105Thr + Ala181Glu
	Thr 64Ser + Ile105Glu + Gln200Asn
	Ser104Asp + Gln200Ser + Thr214Pro
	Thr 64Pro + Ala156Glu + Thr202Gly
15	Gly 63Gln + Gly100Asp + Leu211His
	Asn 60Ser + Gly 98Asp + Leu211Thr
	Tyr161Gly + Val197Cys + Ser206Glu
	Thr207Gln + Tyr208Gln + Ser210Glu
	Gly 98Ser + Ile105Leu + Ser210Glu
20	Thr 64Gly + Pro195Ser + Thr214Ser
	Val 93Met + Ser134Asp + Leu211Met
	Leu124Ser + Asn198Ser + Leu211Asp
	Thr 64Gly + Ala156Ser + Gly157Pro
	Gly 59Ser + Gln200Asn + Gly213Glu
25	Gly 61Asn + Leu124Ile + Ala181Pro
	Ala 96His + Gln185Asp + Leu211Ala
	Ser101Asp + Asn198Ser + Thr214Ser
	Leu 94Met + Asn153Asp + Pro195Gln
	Pro127Gln + Gly152Asp + Val193His
30	Ser 97Asp + Ile105Thr + Thr207Gln
	Gly 61Pro + Ser160Glu + Thr207Gln
	Leu124Val + Pro204Gly + Leu211Ile
	Ser 99Asp + Asn198Gln + Gln200Ser
	Ser101Glu + Ala181Asn + Ala209Pro
35	Gly 61Asn + Ala 96Thr + Gly 98Ser
	Val 93Pro + Asn198Asp + Thr207Gln
	Thr 64Pro + Ala 96Pro + Gly155Glu
	Thr 64Gly + Ala181Thr + Thr214Pro
	Thr 64Pro + Leu 94Cys + Tyr208Met
40	Gln 57Asn + Leu 94Gln + Thr214Asn
	Leu 94Gln + Tyr161Pro + Thr207Gly
	Val 93His + Gln185Asn + Thr207Pro
	Gly125Pro + Asn198Glu + Gln200Ser
	Asn153Ser + Ile159Cys + Ser160Asp
45	Thr 64Gly + Gln200Glu + Leu211Cys
	Leu 94Val + Pro195Gln + Leu211Asp
	Gly 59Pro + Gly100Ser + Thr207Pro
	Tyr102Cys + Gly157Gln + Thr207Pro
	Ser 99Asp + Leu124Ser + Thr214Asn
50	Gly 59Gln + Gln200Asp + Thr207Ser
	Ala 96His + Ala181Asp + Leu211Cys
	Val 93Met + Ala181His + Thr207Ser
	Pro129Gln + Asn198Ser + Thr207Ser
	Asp 58Glu + Gly 95Ser + Ala194Gly

	Ser160Glu + Thr207Gln + Thr214Gly
	Thr 64Asn + Phe183Asp + Thr207Asn
	Ser156Asp + Phe183Ile + Val197His
5	Gly 95Pro + Asn153Ser + Gln200Glu
	Gly157Asp + Pro204Gly + Thr207Ser
	Val197Pro + Asn198Gln + Thr207Gln
	Gly 61Pro + Leu 94Glu + Tyr161Ile
	Pro129Gln + Gly155Pro + Ser210Glu
	Val 93Met + Ser182Asp + Gln200Ser
10	Pro127Ser + Leu211Met + Gly213Ser
	Ala 96His + Ser160Asp + Tyr208Gly
	Gly 98Pro + Pro129Ser + Pro204Asp
	Gly 59Ser + Gly 63Pro + Pro129Asp
	Gly100Pro + Leu124Pro + Thr207Asn
15	Ser103Asp + Ile159Ala + Thr202Gln
	Gly 59Asn + Ser130Glu + Leu211Thr
	Ala 96Gly + Ala131Asn + Gly155Pro
	Asn153Ser + Gln200Asn + Ser210Glu
	Asn153Glu + Thr207Pro + Leu211Ala
20	Pro127Asn + Val197His + Thr207Asp
	Gly100Asp + Ala131Gln + Tyr208Thr
	Gly125Pro + Phe183Pro + Asn198Glu
	Asn 60Ser + Ile105Thr + Thr207Glu
	Thr 64Ser + Pro127Asp + Ala131Thr
25	Ser 99Asp + Leu124Asn + Phe183Pro
	Thr 64Gln + Ser101Asp + Leu211Ser
	Pro195Gln + Val197Gly + Leu211Ser
	Gly100Asn + Tyr102Gly + Thr207Pro
	Asn198Ser + Gln200Asp + Leu211Pro
30	Tyr102Met + Ser182Glu + Leu211Ala
	Gly 61Asn + Gln200Asn + Gly213Asn
	Ser 99Asp + Gly152Pro + Gly157Ser
	Leu124Ser + Tyr161Met + Gln200Glu
	Tyr102Met + Leu211Glu + Thr214Ser
35	Asp 58Glu + Gly 98Asn + Tyr161Asn
	Ile159Ser + Gln200Asn + Tyr208Asn
	Thr 64Asn + Ala156Gly + Leu211Asp
	Gln200Asn + Ser210Glu + Leu211Thr
	Asn 60Asp + Gly155Pro + Val197Asn
40	Ala156Gln + Asn198Glu + Gly205Gln
	Gln 57Asp + Gln200Ser + Tyr208Thr
	Leu124Ile + Ala209Glu + Ser210Asp
	Gly 59Glu + Asn 60Asp + Leu211Ala
	Ala131Gly + Gly152Asp + Asn153Glu
45	Tyr203Val + Ser210Glu + Leu211Glu
	Gly152Asn + Ser210Asp + Leu211Glu
	Phe183Val + Ser210Asp + Leu211Asp
	Tyr102Leu + Ser210Asp + Leu211Glu
	Gln200Asn + Thr207Glu + Tyr208Glu
50	Ala156Ser + Thr207Glu + Tyr208Asp
	Pro129Asp + Ser130Asp + Asn198Ser
	Asn198Ser + Gln200Glu + Ser210Asp
	Leu124Cys + Gln200Glu + Ser210Glu
	Thr 64Gly + Gln200Asp + Ser210Asp

	Gly 61Asn + Gln200Asp + Ser210Glu
	Phe183His + Val197Glu + Asn212Asp
	Ala 96Gly + Ser101Glu + Ser103Glu
	Ser126Glu + Ser128Glu + Asn198Ser
5	Gly 61Pro + Ser154Asp + Ala156Glu
	Val 93Asp + Ser104Asp + Leu211Ala
	Ala 96Ser + Gly125Asp + Tyr161Asp
	Ser 97Asp + Ser 99Glu + Leu211Cys
	Ser 97Asp + Ser 99Glu + Asn153Gln
10	Ala 96Pro + Gly152Asp + Ser154Asp
	Gly 98Asn + Asn198Asp + Gln200Asp
	Asn 60Ser + Asn198Glu + Gln200Asp
	Pro129Ser + Asn198Asp + Gln200Asp
	Gln 57Glu + Gly 59Glu + Leu211Gln
15	Ser128Asp + Ser130Glu + Ala209His
	Ser158Asp + Ser160Glu + Gln185Asp
	Pro127Gln + Ser210Glu + Asn212Asp
	Ser210Glu + Leu211Asn + Asn212Asp
	Val197Asp + Gln200Glu + Ser210Asp
20	Phe183Glu + Tyr208Val + Gly213Asp
	Pro127Glu + Gly152Glu + Tyr161Asp
	Leu 94Glu + Ser101Asp + Leu124Ile
	Leu 94Glu + Ser101Asp + Gln200Asn
	Pro127Gln + Asn153Glu + Ala156Glu
25	Asp 58Glu + Gly 61Gln + Gly 95Asp
	Pro129Gly + Val197Asp + Ser210Glu
	Asp 58Glu + Gly 61Glu + Gly 95Glu
	Val197Glu + Ser210Asp + Gly213Asp
	Ser154Glu + Ser182Glu + Pro204Ser
30	Ser101Glu + Gly125Glu + Ser126Glu
	Ser 99Asp + Ser104Asp + Ile105Leu
	Ser128Glu + Ala131Glu + Gly196Gln
	Gly 61Glu + Ser 97Asp + Ala131His
	Ser 97Asp + Gly100Asp + Ser104Glu
35	Asp 58Glu + Pro204Asn + Gly205Asp
	Gln185Glu + Leu211Cys + Gly213Asp
	Gln200Asp + Thr207Glu + Leu211Asn
	Gly125Ser + Gln200Asp + Thr207Glu
	Gln200Glu + Thr207Asp + Leu211Cys
40	Asp 58Glu + Val 93Gly + Ser 97Glu
	Asp 58Glu + Ser 97Glu + Tyr203Asn
	Gly 61Glu + Gly 95Asn + Thr207Asp
	Ala 96Pro + Thr207Asp + Ser210Asp
	Pro204Asn + Thr207Glu + Ser210Asp
45	Gly100Pro + Thr207Asp + Ser210Asp
	Gly 61Asp + Thr207Asp + Ser210Asp
	Asp 58Glu + Thr 64Asp + Ser 97Glu
	Asn153Asp + Gly157Glu + Ala181Glu
	Ser130Asp + Ser160Asp + Gly205Gln
50	Tyr102Asp + Ser128Asp + Ser160Asp
	Gln 57Asp + Ser206Asp + Thr207Glu
	Ser 99Asp + Ser126Glu + Gly152Gln
	Ala181His + Asn198Glu + Thr214Glu
	Gln 57Asn + Thr 64Glu + Gln200Glu

	Thr 64Asp + Leu124Thr + Gln200Asp
	Gly 98Asp + Gly125Glu + Thr207Gln
	Asn198Glu + Gln200Glu + Pro204Glu
	Asp 58Glu + Gly 59Asp + Gln200Asp
5	Leu124Asp + Val197Asp + Asn198Asp
	Pro129Glu + Asn212Asp + Gly213Glu
	Ser158Asp + Ser210Asp + Leu211Glu
	Ser101Glu + Tyr102Glu + Gln200Asp
	Ser103Asp + Ser104Asp + Asn198Asp
10	Asn 60Glu + Ser103Glu + Ser104Glu
	Ser182Asp + Gln200Asp + Ser210Asp
	Gly157Glu + Gln200Glu + Ser210Asp
	Gln 57Asp + Gln200Asp + Ser210Glu
	Ser 99Glu + Gln200Asp + Ser210Asp
15	Ser182Glu + Asn198Asp + Gln200Glu
	Gln 57Ser + Gln200Asp + Gly213Glu
	Ser 99Glu + Ser103Asp + Ile159Met
	Ser 99Asp + Ser103Asp + Gly213Gln
	Gly155Glu + Gln185Glu + Asn198Glu
20	Gly155Asp + Gln185Glu + Ser210Asp
	Gln 57Asp + Thr 64Glu + Ser210Glu
	Thr207Glu + Leu211Glu + Gly213Asp
	Ser126Asp + Ser160Asp + Ser210Asp
	Ser130Glu + Asn198Asp + Ser210Asp
25	Ser126Asp + Asn198Asp + Ser210Glu
	Ser101Asp + Asn198Glu + Leu211Glu
	Gly155Glu + Asn198Asp + Leu211Glu
	Asp 58Glu + Asn 60Glu + Ser103Asp
	Ser 99Glu + Ser104Glu + Ser126Glu
30	Gly 59Asp + Gly 61Gln + Ser 99Asp
	Asn 60Glu + Ser158Glu + Gln185Glu
	Gly100Glu + Ser104Asp + Ser210Glu
	Gly100Asp + Ser104Asp + Ser182Glu
	Ser128Glu + Ser130Asp + Asn198Glu
35	Ser154Glu + Gln200Asp + Leu211Glu
	Ser103Asp + Gln200Glu + Leu211Asp
	Ser103Glu + Gln200Glu + Leu211Asp
	Ser126Asp + Ala156Glu + Ser182Asp
	Ser158Asp + Ser160Glu + Val197Glu
40	Ser158Asp + Ser160Asp + Val197Glu
	Val 93Gln + Gly125Glu + Ser158Glu
	Gln 57Asp + Leu124Asp + Ser126Asp
	Asn 60Asp + Ser210Glu + Asn212Glu
	Gly157Asp + Ser210Glu + Asn212Asp
45	Ser 99Glu + Ser101Glu + Ser210Glu
	Asp 58Glu + Asn198Glu + Pro204Glu
	Tyr102Asp + Ser130Glu + Ser182Asp
	Asn 60Asp + Gly 95Glu + Ser126Asp
	Leu 94Glu + Ser 97Asp + Ser206Glu
50	Gln 57Asp + Ser126Glu + Gly152Asp
	Leu 94Glu + Ser101Glu + Leu211Asp
	Gly 59Glu + Ser 97Glu + Ser182Glu
	Gly100Asp + Ser103Glu + Ser206Asp
	Asn 60Glu + Gln185Glu + Thr214Glu

	Asn 60Glu + Ser130Glu + Pro204Asp
	Asn 60Asp + Asn198Asp + Pro204Asp
	Ser 99Asp + Ser182Glu + Gln185Asp
	Pro204Asp + Ser210Glu + Leu211Gly
5	Tyr161Val + Pro204Glu + Ser210Glu
	Ser101Glu + Asn198Asp + Gly213Glu
	Leu124Asp + Ser154Glu + Val193Ser
	Ser 99Glu + Ala181Glu + Asn212Asp
	Ala 96Asp + Gly100Asp + Ser154Glu
10	Gly 61Asp + Ser 99Asp + Ser210Glu
	Asp 58Glu + Thr207Glu + Ser210Asp

TABLE 35

Multi-loop Quadruple Mutation Variants	
15	Gln 57Ser + Asn 60Ser + Leu 94Gly + Gln200Glu
	Val 93Gln + Tyr102Cys + Thr207Gly + Gly213Asp
	Ile105Val + Leu124Ile + Ser154Glu + Asn198Gln
	Leu 94Gly + Ser103Glu + Gln200Asn + Ala209Gly
	Ala156His + Gln200Ser + Thr207Pro + Leu211Thr
20	Pro129Asn + Gly155Asp + Ala209Gln + Asn212Gln
	Leu124Pro + Ala194Asn + Asn198Glu + Gln200Asn
	Gly 61Ser + Asn198Gln + Gln200Ser + Leu211His
	Tyr203Thr + Thr207Asn + Tyr208Gly + Asn212Glu
	Gln 57Asn + Asn153Asp + Leu211Ser + Gly213Pro
25	Ala131Asn + Gln185Ser + Val193Ser + Gln200Asn
	Ala181Gly + Ala194Pro + Asn198Ser + Asn212Asp
	Ser101Asp + Leu124Val + Pro195Gln + Thr207Pro
	Gly 95Asp + Asn198Gln + Gln200Ser + Thr207Ser
	Ala 96Gln + Tyr102Pro + Ser182Glu + Val197Asn
30	Asn 60Gln + Gly100Ser + Ser101Asp + Ile159Pro
	Gly 63Asn + Asn153Asp + Gly205Ser + Thr207Ser
	Ser126Glu + Ala131Pro + Asn153Ser + Phe183Thr
	Asn153Gln + Gly205Pro + Ser210Glu + Gly213Pro
	Ile159Glu + Ala194Ser + Pro204Gly + Leu211Ile
35	Asp 58Glu + Val 93Ser + Ala194Pro + Gln200Ser
	Gly 95Asp + Ile105Gln + Phe183Cys + Asn198Gln
	Gly 59Pro + Gly 98Asn + Leu211Val + Thr214Asp
	Gly 61Pro + Val 93Pro + Pro129Asn + Gly213Pro
	Gln 57Asn + Ala156Pro + Ala181Pro + Phe183Gly
40	Gly 95Gln + Gly152Asp + Phe183Met + Asn198Gln
	Gly 59Ser + Gly 95Asn + Pro127Gln + Ser210Glu
	Val197Cys + Asn198Gln + Thr207Glu + Leu211Cys
	Pro129Asn + Ser154Asp + Phe183Pro + Leu211Cys
	Gly 59Gln + Gly100Glu + Ala131Gly + Ala181Gln
45	Gly 61Glu + Tyr161Leu + Pro204Ser + Gly213Pro
	Asp 58Glu + Ile159Met + Tyr161Gln + Gly213Asn
	Ile105Pro + Leu124Asn + Ile159Ser + Asn198Asp
	Tyr102Pro + Ala156Ser + Phe183His + Pro204Asp
	Tyr102Pro + Ile105Pro + Gln200Asn + Pro204Glu
50	Thr 64Ser + Ile105Glu + Gln200Ser + Thr214Pro
	Gly 63Gln + Gly100Asp + Asn198Ser + Leu211His
	Asn 60Asp + Ile105Leu + Gly152Gln + Ala209Gln

Gly152Gln + Gln185Asn + Gly205Ser + Leu211Ala  
 Gln 57Ser + Gly100Pro + Pro127Gly + Asn198Glu  
 Leu 94Cys + Pro127Gly + Val197Gly + Asn198Ser  
 Leu 94Thr + Tyr102Cys + Leu124Cys + Tyr208Ser  
 5 Ala 96Ser + Ala181Asn + Pro204Ser + Tyr208Ile  
 Leu124Val + Asn198Gln + Pro204Gly + Leu211Ile  
 Gly 61Asn + Ala 96Thr + Gly 98Ser + Ser210Asp  
 Thr 64Pro + Ala 96Pro + Gly155Glu + Leu211Cys  
 Gly157Asp + Val197Pro + Asn198Gln + Pro204Gly  
 10 Pro129Gln + Gly155Pro + Val197His + Ser210Glu  
 Val 93Met + Ser182Asp + Gln200Ser + Gly213Ser  
 Leu 94Glu + Pro127Ser + Gln200Asn + Leu211Met  
 Ala 96His + Gly 98Pro + Pro129Ser + Pro204Asp  
 Ser 97Asp + Gly100Pro + Leu124Pro + Thr207Asn  
 15 Gly 59Asn + Thr207Pro + Ser210Glu + Leu211Thr  
 Ala 96Gly + Ala131Asn + Gly155Pro + Ser210Glu  
 Asn153Ser + Val197Thr + Gln200Asn + Ser210Glu  
 Asn 60Ser + Ile105Thr + Ser182Glu + Thr207Ser  
 Gly 98Asn + Tyr161Asn + Gln200Asn + Tyr208Asn  
 20 Leu 94Gly + Ser 99Glu + Leu124Ser + Ala181Pro  
 Ser104Glu + Ala131Pro + Leu211Ser + Gly213Ser  
 Tyr102Asp + Asn198Ser + Thr207Gln + Leu211Asn  
 Gly 98Pro + Ser206Glu + Leu211Gly + Gly213Pro  
 Ala131Asn + Ala181Thr + Tyr208Gln + Ser210Asp  
 25 Gly155Gln + Tyr161Cys + Gln185Ser + Ala209Gln  
 Thr 64Asn + Val 93Asn + Ser126Glu + Gln200Asn  
 Ala131Pro + Phe183Ala + Thr207Asp + Thr214Gln  
 Ser 97Glu + Leu124Pro + Ala131Ser + Pro204Gly  
 Gly 59Asp + Tyr161His + Thr207Gly + Leu211Met  
 30 Gly 95Pro + Gly 98Pro + Ser101Glu + Leu211Cys  
 Gly 59Ser + Thr 64Gly + Leu124Ile + Gly125Glu  
 Thr 64Pro + Ser101Glu + Tyr161Gly + Gln200Asn  
 Gly100Glu + Pro195Ser + Val197Ser + Thr207Asn  
 Ser160Glu + Tyr161Gln + Pro204Ser + Leu211Gln  
 35 Leu124Met + Tyr161Leu + Ser182Glu + Leu211His  
 Ile105Thr + Pro127Asn + Gly157Asn + Tyr161Pro  
 Gln185Asn + Thr207Gln + Asn212Ser + Thr214Gln  
 Val 93Gln + Leu 94Ser + Gly 98Glu + Thr214Gly  
 Asn153Gln + Ser154Glu + Ala181Asn + Leu211Val  
 40 Gly125Asn + Ser154Asp + Val197Ser + Leu211Val  
 Ile159Asn + Gln185Glu + Val197Met + Asn212Gln  
 Ala 96Pro + Gly196Gln + Val197Ala + Asn198Gln  
 Ala131Glu + Gly152Gln + Ala194Ser + Leu211Ile  
 Phe183Ser + Asn198Asp + Gln200Ser + Thr207Pro  
 45 Gly 95Gln + Gly100Ser + Ser130Glu + Thr207Gln  
 Leu124Cys + Gly152Ser + Tyr161Met + Thr207Gln  
 Leu 94Met + Leu124Met + Leu211Ala + Asn212Ser  
 Gly 61Asn + Ala 96Ser + Gly125Pro + Gln200Asp  
 Ile159Gln + Val197Glu + Gln200Asn + Tyr208His  
 50 Tyr161Asn + Ser182Glu + Ala209Ser + Asn212Ser  
 Gln 57Asn + Gly152Ser + Phe183Glu + Gln185Asn  
 Asn198Gln + Thr207Glu + Ala209Gly + Gly213Pro  
 Val197Glu + Tyr208Gly + Ala209Pro + Leu211Pro  
 Ala131His + Gly205Gln + Tyr208Asp + Leu211Gln

	Gly 61Asn + Val 93Cys + Gly 98Gln + Leu211Glu
	Leu124Ala + Val197Gln + Ser210Glu + Asn212Ser
	Pro127Gln + Ile159Leu + Ser160Glu + Ala209Ser
5	Asp 58Glu + Gly 59Gln + Gly205Gln + Leu211Cys
	Asp 58Glu + Phe183Tyr + Asn198Ser + Thr207Gln
	Pro204Gly + Ser206Asp + Thr207Gln + Leu211His
	Gln 57Asp + Gly 98Ser + Ile105Gln + Tyr208Met
	Ser103Glu + Gly155Ser + Ala209His + Leu211Thr
	Ser104Glu + Ala181Asn + Asn198Gln + Leu211Thr
10	Gln185Asn + Val197Cys + Gly205Asp + Leu211Cys
	Gly 59Gln + Tyr102Glu + Pro127Gly + Ala209Thr
	Ala131Thr + Asn153Ser + Val193Gln + Val197Asp
	Ala131Ser + Asn153Ser + Phe183Tyr + Ala194Ser
	Pro129Gln + Ala181Pro + Ala209His + Leu211Ala
15	Pro127Gly + Gly196Asn + Ser210Glu + Leu211Met
	Leu 94Ile + Gly100Asp + Asn198Ser + Tyr208Leu
	Asn153Ser + Gly155Pro + Gly205Asp + Leu211Ser
	Gly 63Asn + Gly152Pro + Asn198Gln + Thr207Glu
	Gly152Ser + Ser160Glu + Tyr161His + Leu211Met
20	Pro129Asn + Asn153Gln + Gly155Gln + Ser210Asp
	Gln 57Ser + Thr 64Gln + Val197Asn + Gln200Asp
	Ser126Asp + Asn153Gln + Pro204Gln + Leu211Gln
	Gly 61Gln + Ala 96Gln + Phe183Leu + Leu211Thr
	Gly 63Asn + Ala156Asn + Asn198Ser + Ser210Glu
25	Gly 59Asp + Gly205Pro + Thr207Gln + Leu211Asn
	Gly 98Ser + Leu124Pro + Ile159Thr + Val197Glu
	Gly205Pro + Ala209Gln + Ser210Glu + Leu211Pro
	Pro129Ser + Thr207Gly + Ser210Glu + Leu211Asn
	Gln 57Asp + Ala131Gln + Leu211Met + Asn212Gln
30	Thr 64Asn + Tyr161Ile + Phe183Asn + Ser210Asp
	Gly155Gln + Pro204Ser + Ala209His + Leu211Asp
	Gly100Gln + Gly157Pro + Ile159Leu + Ser160Asp
	Gly 98Pro + Ser103Asp + Ala131Gly + Leu211Asn
	Gln 57Ser + Gly 61Pro + Ile159Gln + Leu211Asn
35	Gly155Ser + Gln185Asp + Val199Met + Thr207Gly
	Asp 58Glu + Ala 96Gly + Phe183Thr + Thr207Gly
	Val 93Ala + Ser126Glu + Ala131Thr + Thr207Pro
	Thr 64Gln + Gly 98Pro + Val197Thr + Gln200Glu
	Gly152Glu + Asn153Gln + Gly155Pro + Gly213Pro
40	Ser154Glu + Asn198Ser + Gln200Ser + Leu211Ser
	Ile105Thr + Ile159Thr + Phe183Val + Leu211Asp
	Leu124Pro + Ser128Glu + Ile159Met + Leu211Pro
	Gln 57Asp + Asp 58Glu + Leu211Thr + Thr214Ser
	Gly 59Asp + Asn 60Asp + Val197Pro + Val199Ala
45	Ala 96Thr + Leu124Cys + Val197Asp + Asn198Glu
	Thr 64Asn + Ala156Gly + Ser210Asp + Leu211Asp
	Leu124Ser + Gln200Asn + Gly205Asp + Ser206Glu
	Gly 63Pro + Ala156Asp + Gly157Asp + Ile159Gln
	Thr 64Gly + Gly100Glu + Ser101Glu + Leu211Asn
50	Gly 61Asn + Asn153Gln + Gln200Asp + Ser210Asp
	Leu124Ser + Gln200Glu + Ser210Glu + Leu211Gln
	Val197Gln + Gln200Glu + Thr207Ser + Ser210Glu
	Ala209Glu + Ser210Glu + Leu211Glu + Gly213Ser
	Thr207Pro + Ser210Glu + Leu211Asp + Asn212Glu

Ser101Asp + Ser104Asp + Asn198Gln + Leu211Ser  
 Thr 64Asn + Ala 96Asp + Gly 98Asp + Gly213Asn  
 Asn198Glu + Gln200Asn + Ser210Asp + Leu211Thr  
 Tyr161Val + Asn198Glu + Ser210Glu + Gly213Asn  
 5 Val 93Pro + Asn198Glu + Gln200Ser + Ser210Glu  
 Val193Pro + Asn198Asp + Thr207Ser + Ser210Asp  
 Gln185Ser + Asn198Asp + Gln200Asp + Ser210Glu  
 Gly 95Ser + Gly 98Gln + Asn198Asp + Leu211Asp  
 Val 93His + Ala 96His + Gln200Glu + Ala209Asp  
 10 Leu 94Cys + Ile159Leu + Gln200Asp + Ala209Glu  
 Gln 57Asn + Gly 61Asp + Thr 64Asp + Leu 94Val  
 Ala156Gln + Asn198Glu + Gly205Gln + Asn212Asp  
 Tyr102Asp + Ser104Asp + Phe183Gln + Val197His  
 Ala 96Ser + Asn153Ser + Gly155Asp + Ser182Asp  
 15 Ala 96Asn + Asn153Glu + Gln185Glu + Thr214Gly  
 Asp 58Glu + Gly 61Glu + Asn153Ser + Leu211Cys  
 Pro129Ser + Asn198Asp + Gln200Asp + Leu211Pro  
 Gly125Ser + Asn198Asp + Gln200Glu + Leu211Ala  
 Asn198Asp + Gln200Asp + Thr207Pro + Leu211Pro  
 20 Gly 98Pro + Asn198Asp + Gln200Glu + Tyr203Ser  
 Asn198Glu + Gln200Glu + Tyr203Leu + Thr214Asn  
 Gly100Asp + Tyr102Asp + Asn153Ser + Tyr208Leu  
 Thr 64Gln + Thr207Asn + Ser210Glu + Asn212Glu  
 Pro127Gln + Ile159Cys + Ser210Glu + Asn212Asp  
 25 Val 93Ala + Ser 99Asp + Ser101Glu + Ile105Asn  
 Gly100Gln + Gly155Gln + Asn212Asp + Thr214Asp  
 Gly 95Ser + Tyr102Leu + Pro129Glu + Ser160Asp  
 Gly 59Ser + Pro129Asp + Ser160Asp + Leu211Pro  
 Thr 64Pro + Ala 96Thr + Asn153Glu + Ser182Asp  
 30 Asp 58Glu + Gly 61Gln + Gly 95Asp + Ala181Thr  
 Gly 63Pro + Ser 99Asp + Ser101Glu + Ser103Glu  
 Asn153Gln + Val197Asp + Leu211Glu + Thr214Glu  
 Gly152Ser + Ala181Pro + Asn198Asp + Gly213Asp  
 Ser154Glu + Gly155Pro + Ser160Asp + Asn198Gln  
 35 Gly152Asn + Ser154Glu + Ser158Glu + Leu211Asn  
 Asn 60Glu + Leu 94Asp + Tyr208Ala + Gly213Pro  
 Thr 64Asp + Gln200Asp + Ser210Asp + Gly213Pro  
 Ser103Asp + Ser130Glu + Ile159Ala + Thr202Gln  
 Ser103Glu + Ser130Glu + Asn198Gln + Pro204Asn  
 40 Thr207Pro + Ser210Asp + Leu211Glu + Thr214Glu  
 Ser210Glu + Leu211Met + Gly213Glu + Thr214Glu  
 Gly125Ser + Gln200Asp + Ser206Glu + Thr207Glu  
 Ala 96Pro + Gly125Asp + Ser154Asp + Ala156Asp  
 Ser 99Asp + Ser104Asp + Ile105Met + Gln200Ser  
 45 Gly 59Gln + Ser128Asp + Ser154Glu + Ser160Asp  
 Asp 58Glu + Gly 98Asp + Leu124Ser + Tyr161Met  
 Gln185Glu + Asn198Ser + Leu211Cys + Gly213Asp  
 Gly 61Gln + Gly 98Pro + Gln200Asp + Thr207Glu  
 Gly100Gln + Gln200Glu + Tyr203Pro + Thr207Glu  
 50 Val197Pro + Gln200Asp + Thr207Asp + Ala209Gln  
 Asp 58Glu + Val 93Gly + Ser 97Glu + Tyr161Ile  
 Asp 58Glu + Ser 97Asp + Gln200Ser + Tyr208Thr  
 Gly152Asp + Gly157Glu + Val199Ser + Thr207Ser  
 Val197Gln + Pro204Ser + Thr207Asp + Ser210Asp



	Gly125Asn + Prol29Asn + Thr207Glu + Ser210Glu
	Ser101Asp + Ser128Asp + Prol29Glu + Gly152Ser
	Leu 94Ala + Asn198Glu + Gln200Asp + Thr214Glu
	Ala 96Ser + Ile105Glu + Ser126Glu + Ser160Asp
5	Ser130Asp + Ser158Glu + Ser160Asp + Asn198Gln
	Leu124Asp + Phe183Glu + Tyr208Val + Gly213Asp
	Ser101Asp + Ser126Glu + Ala156Gly + Thr207Asn
	Asp 58Glu + Asn 60Asp + Gly100Asp + Ala181Gln
	Asp 58Glu + Gly205Ser + Thr207Asp + Tyr208Glu
10	Thr 64Gln + Leu 94Glu + Ser 97Asp + Gly125Asp
	Ser158Glu + Ile159Asp + Leu211Val + Gly213Glu
	Gln 57Asp + Thr 64Asp + Leu124Thr + Gln200Asp
	Gly 61Glu + Gln200Ser + Gly205Glu + Ser210Glu
	Asp 58Glu + Ile105Gly + Leu211Asp + Asn212Glu
15	Gln 57Asp + Asp 58Glu + Gln200Glu + Pro204Ser
	Gln 57Asp + Asp 58Glu + Ala 96His + Ser160Glu
	Gly157Asp + Gln200Ser + Ala209Glu + Ser210Glu
	Gly 59Glu + Asn 60Asp + Gly 95Asn + Gln185Glu
	Ser101Asp + Tyr102His + Val197Glu + Asn198Glu
20	Gly152Asp + Asn153Glu + Ser210Asp + Leu211Met
	Gly 59Asp + Tyr102Ile + Ser210Asp + Leu211Asp
	Ser101Glu + Tyr102Asp + Gln200Glu + Leu211Asn
	Gly 59Asn + Ser130Glu + Gly205Asp + Ser206Asp
	Ser128Asp + Tyr161Gly + Ser206Asp + Thr207Glu
25	Asn 60Gln + Ser 97Glu + Ser182Asp + Phe183Asp
	Gly 98Glu + Ser 99Asp + Ser160Glu + Thr207Gln
	Gly 98Asp + Thr202Gln + Thr207Glu + Tyr208Asp
	Ser154Glu + Tyr161Leu + Ser210Glu + Asn212Glu
	Asn 60Asp + Ile105Ser + Gln200Asp + Ser210Glu
30	Ala131Asp + Gln200Glu + Ser210Asp + Leu211Pro
	Gln 57Asp + Gln200Glu + Ser210Asp + Gly213Pro
	Prol29Gln + Ser160Glu + Gln200Asp + Ser210Glu
	Prol29Asp + Asn198Gln + Gln200Asp + Ser210Glu
	Gly 59Glu + Thr 64Gly + Gln200Glu + Ser210Glu
35	Asn 60Glu + Ala 96Glu + Asn198Gln + Ser210Glu
	Asn 60Asp + Ala 96Asp + Ser130Glu + Ala156Pro
	Gln 57Ser + Ile159Ser + Gln200Asp + Gly213Glu
	Gly155Asp + Gln185Glu + Asn198Glu + Tyr203His
	Ser104Glu + Leu124Asp + Gln185Ser + Val197Gly
40	Gly 59Gln + Ser101Glu + Ser103Asp + Asn198Asp
	Asn 60Gln + Ser126Asp + Ser128Asp + Ser210Glu
	Ser126Glu + Ser128Asp + Gly155Asn + Asn212Glu
	Ser103Asp + Asn198Glu + Ser210Glu + Thr214Gln
	Ser160Glu + Asn198Glu + Ser210Asp + Leu211Ser
45	Asp 58Glu + Asn198Asp + Thr207Pro + Ser210Asp
	Gly 98Asn + Ser103Glu + Asn198Glu + Ser210Glu
	Ser128Asp + Gly155Pro + Asn198Asp + Ser210Asp
	Gly152Glu + Asn198Asp + Thr207Asn + Ser210Asp
	Ser101Asp + Prol29Gly + Asn198Glu + Leu211Glu
50	Asn198Glu + Tyr208Asp + Gly213Ser + Thr214Ser
	Ser 97Glu + Gly152Glu + Ser154Asp + Gln185Ser
	Thr 64Asp + Ser210Asp + Leu211Val + Gly213Gln
	Thr 64Glu + Gly205Ser + Ser210Asp + Leu211Asn
	Leu 94Thr + Ser126Glu + Pro204Glu + Thr207Asp

Gly100Gln + Tyr102Asp + Pro204Asp + Thr207Asp  
 Ser101Glu + Asn198Glu + Gln200Glu + Leu211Ala  
 Thr 64Asp + Ser 99Glu + Pro204Glu + Thr207Asn  
 Gly155Pro + Ser182Asp + Gln200Asp + Leu211Glu  
 5 Gly100Asn + Ser160Glu + Gln200Glu + Leu211Glu  
 Asp 58Glu + Val 93Asn + Ser206Glu + Leu211Met  
 Ala131Pro + Asn153Glu + Ser182Asp + Asn198Glu  
 Asp 58Glu + Ala 96Glu + Pro127Ser + Gly157Glu  
 Leu 94Asp + Tyr102Leu + Thr207Glu + Ala209Glu  
 10 Ala 96Gln + Ser158Asp + Ser160Glu + Val197Glu  
 Gly100Asp + Ser126Glu + Ser154Asp + Ile159Gly  
 Tyr102Asn + Gln200Glu + Thr207Glu + Gly213Asp  
 Val 93Glu + Pro127Ser + Ser210Asp + Asn212Asp  
 Ile105Asp + Ala131Glu + Ala156Gln + Thr202Pro  
 15 Gln 57Ser + Ser126Glu + Gly152Asp + Ser210Glu  
 Ser126Glu + Gly152Glu + Asn198Glu + Thr207Asn  
 Leu 94Glu + Ser101Asp + Tyr102Leu + Thr207Glu  
 Leu 94Asp + Ser101Asp + Ser154Asp + Gly213Asn  
 Asn 60Asp + Thr 64Glu + Ser103Glu + Thr214Asn  
 20 Asp 58Glu + Val197Asp + Ser210Glu + Leu211Pro  
 Ser160Asp + Phe183Tyr + Val197Asp + Ser210Asp  
 Pro129Glu + Val197Glu + Ser210Glu + Leu211His  
 Ser126Asp + Ser130Glu + Ser182Glu + Gln200Ser  
 Ser126Asp + Ser130Glu + Asn198Asp + Leu211His  
 25 Thr 64Ser + Pro204Asp + Ser210Asp + Leu211Ile  
 Ser 97Asp + Gly100Glu + Gly152Pro + Asn198Glu  
 Ser 97Asp + Gly100Glu + Gln200Glu + Tyr208Gly  
 Asp 58Glu + Gly 59Pro + Ser154Asp + Gly157Asp  
 Gly155Gln + Ala181Glu + Gln185Asp + Ser210Asp  
 30 Gly125Glu + Asn153Asp + Gln200Asp + Tyr208Pro  
 Gln 57Ser + Ser101Asp + Ser154Asp + Ser160Asp  
 Ser104Asp + Asn198Glu + Ala209Asp + Leu211Met  
 Leu124Ile + Gly125Pro + Pro129Glu + Ser158Glu  
 Ser154Glu + Ser158Asp + Gly205Pro + Thr207Glu  
 35 Gly152Glu + Ser158Asp + Gln200Asp + Thr207Pro  
 Ala131Asp + Gly152Asp + Ser158Asp + Val197Thr  
 Ser103Glu + Gln200Glu + Thr207Pro + Asn212Asp  
 Ser130Glu + Gln200Glu + Thr202Ser + Asn212Glu  
 Asn 60Glu + Leu 94Glu + Ile105Leu + Ser130Glu  
 40 Ser 99Glu + Pro127Asp + Pro129Ser + Ser154Glu  
 Asn153Glu + Ile159Gly + Leu211Glu + Gly213Asn  
 Ala156Asp + Ser160Glu + Gly205Gln + Leu211Asp  
 Ser104Asp + Ser130Asp + Gly155Pro + Gly157Asn  
 Gly 98Asp + Ser101Asp + Ser128Asp + Ala181Gln  
 45 Asn 60Asp + Tyr102Gly + Gln200Glu + Thr207Asp  
 Gly 59Pro + Ala131Asp + Ser154Asp + Thr214Asp  
 Ser103Glu + Ser130Glu + Asn198Glu + Asn212Gln  
 Asn 60Asp + Gly 98Asn + Ser103Glu + Ser130Asp  
 Ser160Asp + Phe183Ser + Ser210Glu + Thr214Asp  
 50 Asp 58Glu + Ser 99Asp + Val197Pro + Pro204Asn  
 Asp 58Glu + Gly 98Glu + Gly155Gln + Leu211Asp  
 Thr 64Glu + Leu 94Asp + Gln185Ser + Ser210Asp  
 Asn 60Asp + Ser 99Glu + Ala181Glu + Val197Asn  
 Ser128Glu + Ser206Asp + Ala209Glu + Asn212Gln

	Gly100Asp + Gln185Glu + Leu211Ser + Gly213Glu
	Ser126Glu + Ser154Glu + Asn198Gln + Gly205Glu
	Gly125Glu + Val197Thr + Gln200Glu + Thr207Asp
	Asn 60Glu + Ile105Pro + Ile159Val + Leu211Glu
5	Pro129Asn + Gln200Glu + Pro204Asp + Thr207Gln
	Asn198Ser + Gln200Asp + Tyr203Ile + Pro204Glu
	Gly 98Ser + Ser103Glu + Ala131Glu + Ser210Asp
	Ser103Glu + Ala131Asp + Tyr161His + Ser210Glu
	Gly 61Glu + Gly125Pro + Ser126Glu + Thr207Glu
10	Gly152Asp + Gly157Asp + Pro204Asp + Ala209Thr
	Pro127Asp + Asn153Glu + Pro204Ser + Ser206Asp
	Gln 57Asp + Gln200Ser + Thr207Glu + Ser210Glu
	Ser154Asp + Thr207Glu + Ser210Glu + Thr214Gln
	Ser130Glu + Pro204Asn + Thr207Glu + Ser210Asp
15	Asp 58Glu + Val 93Ala + Ser101Glu + Ser130Asp
	Gly125Glu + Ser130Asp + Gln200Asp + Leu211Thr
	Leu 94Glu + Ser182Asp + Thr207Gln + Asn212Glu
	Ser 97Glu + Ile159Asp + Phe183Ile + Thr214Asp
	Gly 95Glu + Ser103Asp + Pro129Asn + Ala131Pro
20	Asn 60Gln + Gly 61Glu + Ser130Asp + Ser210Asp
	Ser101Glu + Ser126Asp + Ala209Glu + Asn212Ser
	Ser101Glu + Ser126Asp + Tyr161Leu + Gly205Glu
	Asn 60Ser + Gly 98Asp + Ser126Glu + Leu211Thr
	Val 93Glu + Ser 97Glu + Pro129Ser + Gln200Glu
25	Thr 64Asn + Gln200Asn + Thr207Asp + Leu211Asp
	Gly 98Glu + Ser104Asp + Ser126Asp + Phe183Gly
	Gly125Asp + Gly157Asp + Thr207Gln + Gly213Glu
	Leu 94Asp + Ser128Asp + Gly152Asp + Leu211Met
	Ser158Glu + Ser182Glu + Thr207Gly + Tyr208Cys
30	Gln200Asn + Pro204Gln + Ser210Glu + Thr214Glu
	Thr 64Glu + Ala131Asp + Ser210Asp + Leu211Ala
	Asp 58Glu + Gly 59Pro + Gly100Pro + Thr207Asp
	Asn198Gln + Gln200Glu + Ser206Glu + Tyr208His
	Gly 95Pro + Ser126Asp + Ala131Glu + Gln200Glu
35	Ser 97Glu + Ser101Glu + Gln200Asp + Pro204Asn
	Ser 97Asp + Ser101Asp + Gly125Gln + Ser210Asp
	Ala131Gly + Ser154Asp + Tyr161Glu + Ser210Asp
	Ser126Glu + Pro204Asp + Thr207Gly + Ser210Asp
	Gly 98Glu + Ser104Asp + Val197Glu + Asn198Ser
40	Ser104Asp + Ser130Glu + Ala156Thr + Thr207Asp
	Ser104Asp + Ser130Asp + Ala131Thr + Thr207Asp
	Pro127Ser + Ser158Asp + Gln200Ser + Thr214Glu
	Asn 60Asp + Tyr102Ser + Val197Asn + Thr207Asp
	Ser101Asp + Pro127Ser + Ala131Glu + Ala156Glu
45	Asp 58Glu + Ser 99Asp + Gln200Glu + Tyr208Ser
	Asp 58Glu + Ser 99Asp + Leu124His + Asn198Glu
	Gly 61Glu + Thr 64Ser + Val 93Asp + Tyr208Gly
	Asn 60Glu + Gln185Ser + Asn198Ser + Ser206Asp
	Gly 61Pro + Ser158Asp + Ala181Glu + Leu211Asp
50	Tyr161Glu + Gln200Glu + Tyr203His + Pro204Glu
	Gly 61Glu + Gly157Asp + Leu211Met + Asn212Asp
	Gly 59Ser + Gly 98Glu + Tyr102Glu + Ser158Asp

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TABLE 36

Multi-loop Quintuple Mutation Variants					
5	Val 93Gln + Tyr102Cys + Ser154Glu + Asn198Gln + Thr207Gly				
	Leu 94Gly + Leu124Ile + Asn198Gln + Ala209Gly + Ser210Glu				
	Leu124Asp + Ala156His + Gln200Ser + Thr207Pro + Leu211Thr				
	Pro129Asn + Gly155Asp + Gly157Asn + Thr207Ser + Asn212Gln				
	Gly 61Ser + Gly 98Glu + Pro127Asn + Gln200Ser + Thr207Pro				
10	Ala156Gln + Gln185Ser + Gln200Asn + Ser210Asp + Leu211His				
	Gly 63Asn + Tyr102Pro + Ala156Gln + Ser182Glu + Val197Asn				
	Ala 96Pro + Ile105Pro + Thr207Ser + Leu211Ile + Thr214Pro				
	Val 93Asn + Val197Gly + Asn198Gln + Ser210Asp + Leu211Ser				
	Val 93Pro + Leu 94Ile + Ile159Asn + Gln200Ser + Thr207Ser				
15	Gly 61Pro + Gly 95Pro + Ser154Glu + Ala181His + Gly213Pro				
	Gly157Ser + Ser182Glu + Pro195Gln + Thr207Pro + Leu211Ser				
	Ser 97Glu + Tyr102Met + Asn198Ser + Ala209Gln + Leu211Ala				
	Leu124His + Ala131His + Gly157Pro + Asn198Asp + Thr202Ser				
	Gly 95Asp + Ala181Pro + Gln200Asn + Leu211Ala + Gly213Gln				
20	Leu 94Ile + Gly 95Asn + Ser158Glu + Leu211Met + Gly213Ser				
	Gly 98Asn + Gly125Pro + Pro127Gly + Tyr161Ile + Tyr208Met				
	Ala 96Ser + Ser154Glu + Tyr161Gly + Gly196Gln + Val197Gln				
	Leu 94Ser + Gly 95Asn + Pro127Asn + Val197Ser + Thr214Asn				
	Asp 58Glu + Val 93Gln + Leu 94Met + Gln200Asn + Thr207Gln				
25	Gly 95Asp + Gly155Ser + Val197Thr + Thr207Gly + Leu211Pro				
	Gly 98Ser + Ala131Glu + Val197Gln + Gln200Ser + Pro204Gly				
	Gln 57Asn + Val 93Thr + Tyr102Ser + Leu124Pro + Ser126Asp				
	Ser101Asp + Tyr102Cys + Leu124Val + Phe183Gly + Val199Asn				
	Asp 58Glu + Thr 64Gln + Ile105Pro + Gly125Gln + Asn198Ser				
30	Gly155Glu + Tyr161Val + Pro204Gly + Thr207Pro + Leu211Ile				
	Gly100Gln + Ser182Asp + Phe183Asn + Asn198Ser + Thr207Gln				
	Gly 98Glu + Pro129Gly + Gly155Pro + Thr207Gly + Thr214Gly				
	Leu 94Met + Gly100Ser + Gly196Gln + Gln200Asn + Asn212Asp				
	Ser104Glu + Gly152Gln + Asn153Gln + Ala194Gln + Asn212Ser				
35	Pro129Gln + Val197Thr + Tyr208Ile + Ser210Glu + Thr214Ser				
	Val 93Gly + Ala 96Asn + Pro129Gln + Ser160Asp + Pro204Gly				
	Pro127Gln + Ala131Asn + Gly196Ser + Val197Thr + Ser206Glu				
	Pro127Ser + Ser126Asp + Ala156His + Ala194Gln + Leu211Ala				
	Gly 95Gln + Pro127Gly + Val197Gly + Gly205Asn + Ser210Glu				
40	Ala131His + Pro204Gly + Thr207Asn + Leu211Thr + Gly213Asn				
	Leu 94Gly + Tyr102Gln + Phe183Val + Thr207Ser + Leu211Asp				
	Gly 61Asn + Ile105Met + Gly125Ser + Val193Gly + Asn198Glu				
	Gly 59Pro + Ala 96Gly + Ser101Asp + Tyr208His + Leu211His				
	Tyr102Val + Gln200Asp + Pro204Ser + Leu211Ile + Thr214Pro				
45	Val 93Thr + Ser104Glu + Pro195Ser + Val199Pro + Leu211Val				
	Ile159Gln + Tyr161Ala + Phe183Leu + Ser210Glu + Thr214Gly				
	Gly 59Asn + Pro127Ser + Ile159Ala + Gln200Asn + Leu211Met				
	Gln 57Glu + Val 93His + Gly125Gln + Ala131Thr + Ala156Asn				
	Ser126Asp + Gly157Ser + Asn198Gln + Gln200Ser + Thr207Ser				
50	Gly125Asn + Pro129Ser + Phe183Asp + Thr207Gly + Leu211Gly				
	Asn 60Asp + Leu 94Met + Tyr102Pro + Gly152Asn + Thr207Gln				
	Ile105Leu + Gly152Pro + Ser158Glu + Asn198Ser + Thr214Gly				
	Ala 96Asn + Ala131Thr + Gly157Asn + Thr207Gly + Ser210Glu				
	Val 93Gln + Leu124Met + Ala181Gln + Asn198Glu + Gln200Ser				
	Pro127Ser + Ile159Thr + Pro195Gly + Asn198Ser + Gln200Glu				

Ala 96Asn + Val197His + Asn198Ser + Gln200Asn + Asn212Asp  
 Asp 58Glu + Leu 94Ala + Leu124Pro + Ala156Gly + Leu211Gln  
 Ile105Thr + Gly157Pro + Phe183Pro + Val197Asp + Tyr208Val  
 Gly 95Glu + Gly100Gln + Tyr102Gly + Tyr161His + Tyr208Cys  
 5 Thr 64Gly + Val 93Asn + Gly 95Pro + Gly100Pro + Asn198Gln  
 Gln 57Asn + Asn 60Gln + Ser101Glu + Gly155Ser + Pro204Ser  
 Gly125Ser + Gln185Asn + Asn198Gln + Ser206Glu + Ala209His  
 Gly 98Ser + Pro129Asn + Val197Glu + Gln200Asn + Tyr208Asn  
 Gly 98Asp + Asn153Gln + Gln200Asn + Pro204Ser + Leu211Ser  
 10 Ala 96Thr + Ala181Thr + Val197Asn + Gln200Asn + Thr207Pro  
 Gly 95Gln + Pro129Asn + Tyr161Ala + Gln185Asp + Thr207Asn  
 Gly 95Asn + Gly152Gln + Ser160Glu + Ala194Asn + Tyr208His  
 Gln 57Asp + Phe183Leu + Thr207Ser + Ala209Gln + Leu211Met  
 Ile105His + Gly125Asp + Gly152Asn + Pro204Asn + Tyr208Leu  
 15 Gln 57Asn + Leu 94Glu + Ala 96Gln + Pro127Gly + Leu211Pro  
 Asn153Ser + Tyr161Met + Ala181Gln + Asn198Ser + Ser210Asp  
 Gly 95Asn + Gly 98Glu + Pro129Asn + Ala209Pro + Thr214Pro  
 Gly 61Asn + Asn198Ser + Ala209Glu + Leu211Ser + Gly213Pro  
 Ser 99Asp + Pro127Asn + Pro195Asn + Tyr208His + Leu211Cys  
 20 Asn 60Gln + Thr 64Asn + Gly100Asn + Ile105Leu + Pro127Asn  
 Pro127Glu + Ala194Gln + Gln200Asn + Thr207Ser + Leu211Cys  
 Thr 64Gly + Leu 94Gly + Val197Asn + Gln200Ser + Ala209Pro  
 Gly 59Gln + Leu 94Ser + Ala 96Ser + Leu211Thr + Thr214Glu  
 Pro129Gly + Ala194Asn + Gln200Ser + Leu211Gln + Asn212Asp  
 25 Ala131Gly + Asn153Glu + Thr207Asn + Leu211Thr + Gly213Gln  
 Gly 59Pro + Thr 64Asn + Asn198Gln + Ala209Glu + Ser210Asp  
 Gly155Asn + Ser160Asp + Tyr161Glu + Asn198Ser + Leu211Ser  
 Ser126Glu + Pro127Glu + Gln200Ser + Thr207Ser + Leu211Pro  
 Ala131Gly + Ala209Thr + Ser210Asp + Leu211Glu + Thr214Gly  
 30 Gly 61Asn + Ile159Ser + Ser206Glu + Thr207Asp + Leu211Ala  
 Gly 98Glu + Ser 99Glu + Tyr161Leu + Phe183Gln + Asn198Gln  
 Gln 57Ser + Val 93Asn + Gly155Asn + Gln200Glu + Ser210Glu  
 Thr 64Asn + Gly 95Glu + Ala 96Asp + Gly 98Asp + Gln200Ser  
 Ile105Ser + Ser210Glu + Leu211Asp + Asn212Asp + Gly213Ser  
 35 Ala 96Asn + Gly100Ser + Ile105Cys + Asn198Asp + Ser210Glu  
 Ala 96Gln + Gly 98Asn + Asn198Asp + Pro204Asn + Ser210Asp  
 Gly 98Gln + Asn198Glu + Gln200Glu + Tyr208Asn + Ser210Asp  
 Ile105Pro + Leu124Asn + Ile159Ser + Asn198Asp + Leu211Asp  
 Leu 94Gln + Tyr161Pro + Asn198Glu + Thr207Gly + Leu211Glu  
 40 Tyr161Gly + Val197Ala + Asn198Gln + Pro204Glu + Ser206Asp  
 Leu124Ala + Asn198Asp + Tyr208Gly + Ser210Glu + Asn212Asp  
 Gln185Asn + Asn198Asp + Gln200Glu + Thr207Gln + Leu211Asp  
 Pro127Gly + Tyr161Ala + Pro204Glu + Thr207Asp + Thr214Ser  
 Gln 57Asn + Leu124Ser + Val197Glu + Asn198Glu + Gln200Glu  
 45 Asp 58Glu + Ala 96Asp + Val197Ala + Gly205Ser + Thr207Gly  
 Ser158Asp + Ser160Asp + Pro195Ser + Thr207Ser + Leu211Met  
 Asp 58Glu + Asn 60Glu + Thr 64Glu + Gly157Asn + Leu211Ala  
 Leu124Glu + Ser126Asp + Ile159Met + Phe183Val + Val193His  
 Leu 94Ile + Pro127Glu + Pro129Asp + Ser130Asp + Tyr203His  
 50 Leu 94Cys + Ser 99Asp + Ser101Glu + Asn198Gln + Asn212Gln  
 Gly 61Asn + Ser128Glu + Ser160Glu + Ala181Asn + Thr207Gln  
 Tyr161Met + Val197Asp + Gln200Asp + Ser210Glu + Asn212Gln  
 Val 93Ala + Gly125Pro + Ser154Glu + Gly157Asp + Ser158Asp  
 Leu 94Glu + Ser101Asp + Phe183Ala + Gln200Asn + Leu211Met

Leu 94Asp + Ser103Glu + Ser104Asp + Asn198Ser + Tyr208Gly  
 Gly125Asp + Asn153Glu + Ser160Glu + Tyr161Asn + Asn198Ser  
 Leu 94Asn + Pro127Asn + Ser154Asp + Gln185Glu + Thr214Asp  
 Gly 61Gln + Thr 64Asn + Asn198Glu + Ser210Asp + Gly213Asp  
 5 Leu124Gln + Asn198Glu + Gln200Asp + Thr207Gly + Tyr208Glu  
 Leu 94Glu + Leu124Asp + Pro129Asn + Ala156Ser + Thr207Gly  
 Gly 98Asn + Ala156Pro + Asn198Glu + Gln200Asn + Gly213Asp  
 Gly 95Glu + Gly 98Gln + Ser 99Glu + Tyr102Cys + Ser104Glu  
 Gly125Asn + Gln200Asp + Thr207Glu + Ser210Asp + Leu211His  
 10 Gly 59Asp + Ser 97Asp + Ser 99Asp + Ala131Pro + Pro204Gly  
 Gln 57Ser + Tyr102Gln + Asn198Glu + Ala209Glu + Leu211Ser  
 Asn153Asp + Ile159Asp + Tyr161His + Phe183His + Gly213Pro  
 Gly100Ser + Gly155Asp + Ile159Glu + Val197Met + Thr207Pro  
 Pro127Ser + Gln200Asp + Thr207Gly + Ser210Asp + Gly213Glu  
 15 Ala 96Thr + Ser126Glu + Gly152Glu + Ser158Glu + Thr207Asn  
 Leu 94Ala + Gly 95Asp + Ser 97Asp + Ser101Asp + Thr202Gly  
 Gly152Ser + Gly205Ser + Thr207Glu + Ser210Glu + Leu211Asp  
 Tyr161Pro + Pro195Ser + Thr207Asp + Ser210Glu + Leu211Asp  
 Asp 58Glu + Ser 97Glu + Ser 99Asp + Gln185Asn + Gly196Ser  
 20 Thr 64Gly + Ser101Glu + Ile105Glu + Ser126Asp + Tyr161Leu  
 Thr 64Gly + Ser 99Asp + Ile105Glu + Gly205Pro + Leu211Ala  
 Asn153Glu + Ala194Thr + Asn198Glu + Tyr208Ile + Gly213Glu  
 Gly152Gln + Gln200Asn + Ser206Glu + Ala209Asp + Ser210Glu  
 Ser103Glu + Gly125Pro + Ala131Glu + Tyr161Cys + Leu211Thr  
 25 Gly100Ser + Gln200Asn + Thr207Glu + Ser210Asp + Leu211Gln  
 Gly100Pro + Asn198Asp + Gln200Glu + Gly205Gln + Thr207Glu  
 Ala 96Asp + Ser 99Glu + Ser104Asp + Val197Met + Gly213Ser  
 Gly 98Asp + Gly100Pro + Ser101Asp + Gly125Ser + Ala156Ser  
 Gly 98Asp + Gly125Asp + Ser160Glu + Tyr161Ile + Thr214Gly  
 30 Gly 61Glu + Gly155Gln + Asn198Gln + Ser206Glu + Tyr208His  
 Asp 58Glu + Asn 60Glu + Leu124Asn + Ser206Asp + Thr207Asn  
 Gly 61Asp + Thr202Pro + Thr207Gln + Ser210Glu + Leu211Pro  
 Gly 61Glu + Tyr102His + Phe183His + Gln185Asn + Ser210Asp  
 Ser 99Asp + Ser126Asp + Pro129Asp + Gly152Ser + Leu211His  
 35 Leu 94Ile + Gly125Gln + Ser128Asp + Pro129Glu + Ser154Asp  
 Asn198Asp + Gln200Ser + Gly205Pro + Gly213Ser + Thr214Asp  
 Gly 61Asn + Ser182Asp + Val193Gln + Asn198Asp + Ser210Glu  
 Gly 61Glu + Leu 94Cys + Gln200Ser + Gly205Glu + Ser210Glu  
 Asp 58Glu + Phe183Ser + Pro204Glu + Thr207Ser + Ser210Glu  
 40 Leu 94Met + Ala156Asp + Tyr161Cys + Asn212Glu + Thr214Glu  
 Asp 58Glu + Leu124Pro + Ile159Thr + Leu211Asp + Asn212Glu  
 Gln 57Glu + Leu 94Thr + Val197Ser + Ala209Glu + Ser210Asp  
 Ser104Glu + Pro129Ser + Ala156Gly + Ala209Glu + Ser210Glu  
 Asn153Asp + Ser154Glu + Asn198Asp + Thr207Gln + Leu211Gln  
 45 Gly 59Asp + Asn 60Glu + Ser101Glu + Tyr102His + Thr207Asn  
 Gly 61Gln + Ser104Asp + Ile105Asp + Asn153Ser + Thr207Asp  
 Ser 97Glu + Val197Gln + Asn198Gln + Asn212Asp + Gly213Asp  
 Tyr102Val + Ser154Glu + Gly205Glu + Ser206Asp + Leu211Cys  
 Asn153Glu + Tyr161Thr + Pro195Gly + Ser206Asp + Thr207Glu  
 50 Gly 59Gln + Thr 64Pro + Gly 98Glu + Ser130Asp + Ala131Glu  
 Gly125Pro + Gly152Asn + Ser158Glu + Ile159Glu + Ser210Glu  
 Ser 97Asp + Gly 98Asp + Ile105Val + Leu124Thr + Gln200Asp  
 Gly100Glu + Ser101Asp + Ser160Asp + Val197Ala + Leu211Gln  
 Ser103Asp + Ser104Asp + Ala161Ser + Asn198Asp + Tyr208Ile

Ser 99Glu + Gly100Glu + Gly125Asn + Ser182Asp + Thr207Gly  
 Leu 94Gly + Gly100Asp + Ala131Gly + Gln200Glu + Ser210Asp  
 Ser 97Glu + Ala131Gln + Gln200Asp + Thr207Gly + Ser210Glu  
 Gly125Asn + Ser130Glu + Phe183Asn + Gln200Asp + Ser210Glu  
 5 Gly 95Gln + Tyr102Leu + Pro127Glu + Gln200Asp + Ser210Asp  
 Gly125Glu + Gly155Asn + Gln200Glu + Ser210Asp + Thr214Gly  
 Gln 57Ser + Asp 58Glu + Gln200Asp + Thr207Gly + Ser210Glu  
 Pro127Asp + Tyr161Cys + Gln185Asn + Gln200Asp + Ser210Asp  
 Gly100Glu + Gln200Asp + Thr207Pro + Ser210Asp + Leu211Ala  
 10 Gly 59Pro + Ser160Asp + Asn198Gln + Gln200Asp + Ser210Glu  
 Thr 64Glu + Ile105Cys + Leu124Ser + Val197Glu + Ser210Asp  
 Gln 57Glu + Val 93Glu + Gly 95Ser + Ser103Glu + Asn198Gln  
 Asn 60Glu + Ala 96Asp + Tyr102Glu + Gln200Ser + Thr207Gly  
 Gln 57Ser + Asn 60Ser + Leu 94Gly + Gln200Glu + Gly213Asp  
 15 Asn198Asp + Pro204Gln + Ser206Glu + Ser210Glu + Leu211Thr  
 Val 93Glu + Ser126Glu + Ser160Asp + Val197Gln + Leu211Ser  
 Gly 98Asp + Ser126Glu + Pro129Asp + Ala181Ser + Gly213Ser  
 Ala 96Glu + Gly 98Glu + Ser103Asp + Pro127Ser + Ile159Pro  
 Gly 63Gln + Ser128Glu + Gly155Ser + Asn198Glu + Ser210Glu  
 20 Gln 57Asp + Ala 96Thr + Gln185Asn + Asn198Asp + Ser210Asp  
 Asn 60Gln + Gly 95Asp + Asn198Glu + Gln200Asn + Ser210Asp  
 Ser104Asp + Ile105Met + Asn198Asp + Ser210Asp + Leu211Asn  
 Tyr102Glu + Asn198Asp + Gln200Asn + Ser210Asp + Leu211Val  
 Gln 57Asp + Gly125Ser + Asn198Asp + Gly205Asn + Ser210Asp  
 25 Tyr102Leu + Gln185Glu + Asn198Glu + Gln200Ser + Ser210Glu  
 Gly 59Asp + Tyr102Ile + Asn198Glu + Ser210Asp + Thr214Ser  
 Val 93Thr + Leu124Val + Ile159Glu + Asn198Asp + Ser210Glu  
 Ser101Asp + Pro129Gly + Asn198Glu + Thr207Ser + Leu211Glu  
 Gly152Gln + Gly155Gln + Asn198Asp + Gly205Asp + Leu211Asp  
 30 Gly 59Gln + Thr 64Ser + Ala156Asp + Ser158Glu + Gly205Glu  
 Pro127Glu + Asn198Ser + Pro204Glu + Ser206Asp + Thr207Pro  
 Asp 58Glu + Ile159Gly + Asn198Asp + Leu211Ile + Asn212Glu  
 Ser130Asp + Ile159Gly + Asn198Glu + Leu211Met + Asn212Glu  
 Asp 58Glu + Asn 60Glu + Ile159Met + Tyr161Gln + Gln200Asp  
 35 Ser 97Asp + Ser103Glu + Ile105Glu + Val197Cys + Leu211Val  
 Ser103Glu + Ile105Glu + Asn153Ser + Gln200Ser + Ser210Asp  
 Ala 96Pro + Gly152Asp + Ser160Asp + Thr207Glu + Leu211Gln  
 Gly 95Asp + Ser 99Glu + Ile105Gly + Gly157Pro + Leu211Glu  
 Ser 97Asp + Ser 99Glu + Val197Glu + Asn198Ser + Thr214Gly  
 40 Gln 57Asn + Ser 97Glu + Ser 99Glu + Asn153Glu + Val193His  
 Thr 64Glu + Ala131Asn + Tyr208Leu + Ser210Asp + Gly213Ser  
 Gln 57Glu + Ala156Pro + Ser158Asp + Gln185Glu + Thr207Gln  
 Ser 97Asp + Pro129Asn + Ser158Glu + Gln185Glu + Leu211Ser  
 Ala 96Thr + Ala156His + Ser158Glu + Gln185Asp + Leu211Asp  
 45 Leu 94Ala + Ser101Asp + Ser126Glu + Ala131Gln + Ser154Asp  
 Gly157Glu + Ile159Leu + Gln200Asn + Pro204Asp + Thr207Glu  
 Leu 94Thr + Ser126Glu + Pro204Glu + Thr207Asp + Gly213Asn  
 Gly100Gln + Ser154Glu + Asn198Glu + Gln200Asp + Leu211Ser  
 Gln 57Glu + Asn198Glu + Gln200Glu + Tyr203Val + Gly213Pro  
 50 Ile105Met + Ser156Glu + Ile159Ser + Asn198Glu + Gln200Asp  
 Ser104Asp + Ala181Gln + Asn198Glu + Gln200Asp + Thr207Gly  
 Gly100Asn + Gly152Pro + Ser182Asp + Gln200Asp + Gly213Asp  
 Tyr102Met + Ser128Glu + Ser130Asp + Asn198Asp + Leu211Met  
 Ser128Glu + Ser130Glu + Ala131Thr + Asn198Glu + Asn212Gln

Val 93Thr + Leu124Glu + Ile159Asn + Gln200Glu + Leu211Asp  
 Gly157Ser + Ser182Asp + Gln200Glu + Thr207Pro + Leu211Glu  
 Ser130Glu + Gln200Asp + Gly205Gln + Thr207Gln + Leu211Glu  
 Gln 57Asn + Gly 59Asn + Asn 60Glu + Gly 98Glu + Ser130Asp  
 5 Gly 61Asp + Tyr102Pro + Ser206Glu + Thr207Asn + Ser210Glu  
 Thr 64Asn + Ser 97Asp + Pro129Asn + Val197Asp + Leu211Glu  
 Ser 99Glu + Ala181Thr + Val197Glu + Ala209Asn + Leu211Asp  
 Gly 59Asp + Val 93Ser + Asn153Ser + Val197Glu + Leu211Asp  
 Ser101Glu + Asn153Ser + Tyr208Met + Leu211Glu + Gly213Asp  
 10 Gly157Asp + Gln185Asn + Thr207Glu + Ala209Asp + Asn212Gln  
 Gly100Gln + Gly125Ser + Phe183Asp + Ser210Asp + Thr214Glu  
 Asn198Gln + Ser206Glu + Thr207Pro + Ser210Asp + Asn212Asp  
 Ala181Thr + Gln200Glu + Thr207Asp + Leu211Ala + Gly213Glu  
 Asn 60Asp + Gly 98Pro + Ser126Glu + Pro129Glu + Gln200Ser  
 15 Asp 58Glu + Ile159Pro + Ser210Glu + Asn212Asp + Thr214Gln  
 Asp 58Glu + Gly125Ser + Ala131Ser + Ser210Asp + Asn212Glu  
 Gly 59Ser + Ser101Glu + Ala131His + Ser210Glu + Asn212Glu  
 Asn 60Gln + Val 93Ala + Ser103Glu + Ser210Glu + Asn212Glu  
 Ser158Glu + Asn198Ser + Tyr208Glu + Ser210Asp + Leu211Ser  
 20 Ala 96Ser + Ser160Asp + Asn198Glu + Asn212Ser + Thr214Glu  
 Leu 94Asn + Gly 98Asp + Gly100Glu + Pro127Glu + Val197Met  
 Asn 60Ser + Gly 98Asp + Gly100Asp + Tyr102Asn + Gln200Glu  
 Leu 94Ser + Ala 96Thr + Ile105Asp + Tyr161Glu + Leu211Ile  
 Gly 95Ser + Ser 99Asp + Ser101Asp + Leu211Met + Gly213Glu  
 25 Gly 61Glu + Gly125Ser + Ser128Glu + Ser160Asp + Ala181Pro  
 Ser182Glu + Val193Met + Asn198Glu + Gly205Gln + Ala209Asp  
 Leu 94Glu + Gly100Asn + Ser104Asp + Ile105Val + Ser206Glu  
 Thr 64Asp + Ser126Asp + Gly152Asp + Pro204Gly + Leu211Asn  
 Gly 59Asp + Val 93Glu + Leu 94Pro + Ile105Met + Leu211Ser  
 30 Pro129Glu + Ser160Asp + Gln200Ser + Gly205Gln + Ser210Glu  
 Ile159Val + Ser160Glu + Phe183Gln + Pro204Gln + Ala209Asn  
 Leu 94Asp + Val197Asp + Thr207Gly + Ser210Asp + Leu211Met  
 Leu124Glu + Phe183Pro + Val197Glu + Tyr208Leu + Ser210Glu  
 Leu 94Met + Leu124Asp + Asn153Asp + Tyr161Ala + Leu211Glu  
 35 Gly 98Asp + Gly152Glu + Ala156Asp + Tyr208Met + Asn212Ser  
 Val 93Asp + Gly 98Gln + Ser126Asp + Ser130Glu + Ala209Pro  
 Asp 58Glu + Tyr102Ala + Ser126Asp + Ser130Glu + Ile159Asn  
 Ser126Asp + Ser130Glu + Val197Gly + Leu211Glu + Asn212Ser  
 Ser104Glu + Ile105Pro + Asn198Asp + Leu211His + Gly213Asp  
 40 Ser 97Asp + Gly100Glu + Gly152Pro + Tyr161Glu + Ala181Gln  
 Ser128Glu + Ala131Glu + Ser154Asp + Asn198Gln + Ala209His  
 Ser 99Asp + Pro129Ser + Ser154Glu + Ser160Asp + Pro204Gln  
 Asp 58Glu + Ile105Cys + Leu124Ser + Ser154Glu + Ser158Glu  
 Ala131Asp + Val197Glu + Gln200Glu + Thr207Pro + Thr214Gly  
 45 Ser 99Asp + Val197Asp + Asn198Gln + Gln200Glu + Thr207Gly  
 Ser101Glu + Val197Asp + Gln200Glu + Pro204Gly + Thr207Ser  
 Ala131Asp + Val197Asp + Asn198Gln + Gln200Glu + Ala209Asn  
 Gly 95Pro + Gly 98Asp + Leu124Glu + Thr207Asn + Asn212Glu  
 Gln 57Glu + Asn 60Glu + Val 93Thr + Gly 95Asn + Ser182Asp  
 50 Asn 60Asp + Ile105Gln + Ala131Thr + Thr207Ser + Ala209Glu  
 Ser103Glu + Gln200Glu + Thr207Pro + Ala209Gln + Asn212Asp  
 Gly 61Pro + Leu 94Glu + Gln200Asp + Leu211Val + Asn212Glu  
 Gly 59Pro + Val 93Glu + Gln200Asp + Leu211Val + Asn212Asp  
 Ser 97Glu + Gly157Ser + Ala181Pro + Gln200Glu + Asn212Glu



Val 93Thr + Gly 98Gln + Tyr102Glu + Ala131Glu + Ser210Asp  
 Gly 61Glu + Ser104Asp + Phe183Ala + Thr207Gly + Ala209Glu  
 Ala156Asp + Ser160Asp + Ala181Gly + Asn198Ser + Gln200Asp  
 Gln 57Glu + Gly 61Asp + Asn153Gln + Asn198Asp + Gly205Ser  
 5 Gly100Glu + Ile105Ser + Leu124Glu + Val197Met + Ser210Asp  
 Tyr102Glu + Ser126Glu + Val193Pro + Pro204Asn + Ser210Asp  
 Gly 59Pro + Ala131Asp + Asn153Ser + Ser154Asp + Thr214Asp  
 Ser103Glu + Ser130Glu + Gln200Ser + Gly205Glu + Thr214Gly  
 Asp 58Glu + Ala156Pro + Gly157Asp + Ser160Asp + Gln200Ser  
 10 Thr 64Glu + Leu 94Asp + Ala 96Gln + Gln185Ser + Ser210Asp  
 Ser130Asp + Gly157Glu + Phe183Glu + Asn198Gln + Leu211Met  
 Asp 58Glu + Pro129Glu + Ala156His + Pro204Ser + Gly205Glu  
 Ser126Glu + Ile159Leu + Ala209Asp + Leu211Ala + Asn212Glu  
 Gly 59Asp + Ser126Asp + Ser154Glu + Phe183Leu + Leu211Val  
 15 Gly155Asn + Gly157Asp + Gln200Glu + Thr207Glu + Leu211Asn  
 Gly 59Glu + Gly 95Pro + Ala181His + Gln200Glu + Thr207Asp  
 Gln 57Glu + Pro127Gly + Gly152Ser + Gln200Asp + Thr207Asp  
 Ala 96Glu + Asn198Ser + Gln200Glu + Thr207Glu + Thr214Ser  
 Thr 64Asp + Leu124Ser + Pro129Asp + Tyr161Asn + Thr207Asp  
 20 Ser 97Asp + Tyr161Gly + Ser210Glu + Leu211Pro + Gly213Asp  
 Pro127Ser + Pro129Asn + Gly157Asp + Ser210Asp + Gly213Glu  
 Tyr102Cys + Ser130Asp + Pro204Gln + Ser210Glu + Gly213Asp  
 Ser 99Glu + Ala156Ser + Ala209Thr + Ser210Asp + Gly213Asp  
 Gly196Pro + Gln200Asp + Pro204Asp + Thr207Gln + Leu211Asn  
 25 Asp 58Glu + Ser 97Glu + Ser104Asp + Pro129Gly + Tyr161Thr  
 Asp 58Glu + Leu 94Ser + Ser 97Asp + Gly 98Pro + Gln200Asp  
 Gly 61Glu + Ser101Asp + Thr207Asp + Leu211Ile + Thr214Ser  
 Gly 61Asp + Ser101Glu + Tyr102Val + Thr207Glu + Thr214Ser  
 Gly 61Asp + Asn153Gln + Ile159Gly + Asn198Asp + Thr207Glu  
 30 Gly 61Glu + Ile159Ser + Val197Met + Asn198Glu + Thr207Asp  
 Thr 64Gln + Gly100Glu + Gln200Asn + Thr207Glu + Ser210Asp  
 Ser126Glu + Gly155Pro + Tyr161Thr + Thr207Glu + Ser210Asp  
 Tyr102Glu + Gly125Gln + Ala181Pro + Thr207Asp + Ser210Glu  
 Gly100Glu + Gly205Ser + Thr207Glu + Ser210Glu + Asn212Gln  
 35 Gly 59Ser + Gly 95Glu + Thr207Glu + Ser210Asp + Leu211Val  
 Tyr161Asp + Asn198Ser + Thr207Glu + Ser210Glu + Gly213Pro  
 Pro129Gln + Ser130Glu + Asn198Gln + Thr207Asp + Ser210Glu  
 Gln 57Asp + Phe183Gly + Pro204Gln + Thr207Glu + Ser210Asp  
 Asn 60Gln + Gly157Glu + Pro204Gly + Thr207Asp + Ser210Asp  
 40 Pro129Asp + Gly152Pro + Thr207Asp + Ser210Glu + Leu211Asn  
 Gly 61Glu + Ser 99Asp + Gly155Asn + Pro195Gln + Gln200Asn  
 Gly 98Glu + Ser101Glu + Ala181Gly + Gln185Glu + Thr207Pro  
 Gly 59Pro + Thr 64Asp + Pro129Gln + Ala181Glu + Asn198Asp  
 Gly 98Asp + Tyr161Met + Ala181Glu + Asn198Asp + Leu211Val  
 45 Ile159Glu + Val197Thr + Tyr208Gln + Ala209Glu + Thr214Asp  
 Ser126Glu + Gly157Glu + Ser182Glu + Asn198Gln + Leu211Val  
 Gly 61Asp + Ser126Glu + Ser206Asp + Tyr208Gly + Leu211Met  
 Gly 98Pro + Ser130Glu + Ser160Glu + Thr207Gly + Ser210Asp  
 Gln 57Asp + Gly125Gln + Ser130Asp + Asn153Ser + Ser160Asp  
 50 Tyr161Met + Gln200Glu + Ser206Glu + Ala209His + Gly213Asp  
 Gly 61Asp + Ala156Glu + Gly205Asn + Thr207Gln + Ser210Glu  
 Ile105Glu + Ser130Asp + Gln200Asn + Gly205Glu + Thr207Asn  
 Gln 57Ser + Asn 60Asp + Gln200Asp + Gly205Glu + Thr207Ser  
 Ser101Glu + Ser126Glu + Val197Asp + Thr207Gly + Leu211Cys

	Ser101Asp + Ser126Asp + Gly152Ser + Asn198Glu + Gln200Asn
	Gln 57Asp + Gly125Asp + Gly205Asp + Thr207Ser + Tyr208Thr
	Leu 94Met + Ser128Asp + Ser158Glu + Asn198Gln + Ser210Asp
	Gly 59Asp + Gly 95Pro + Ala194Gln + Asn198Asp + Thr214Asp
5	Gln 57Glu + Gly 61Asn + Gly 98Asp + Gly100Pro + Val193Cys
	Thr 64Gln + Val 93Glu + Ser 97Glu + Pro129Ser + Gln200Glu
	Gln 57Glu + Thr 64Asn + Val 93Asp + Gln200Glu + Thr207Asn
	Gly100Asp + Gly125Ser + Ser126Glu + Gly196Gln + Val197Glu
	Val 93Met + Gly100Asp + Ser126Glu + Tyr161Ala + Asn198Asp
10	Gly100Glu + Ser126Asp + Ala181His + Val197Cys + Gln200Glu
	Asn 60Gln + Ser 99Asp + Ser103Glu + Ser210Asp + Leu211Ala
	Gln 57Ser + Ser 99Glu + Ser103Glu + Gln200Asp + Thr207Asn
	Gly 63Ser + Ala 96Ser + Ser210Glu + Leu211Pro + Thr214Asp
	Pro129Gly + Val197Gln + Ser210Glu + Leu211His + Thr214Asp
15	Pro127Asp + Gly155Glu + Tyr161Ser + Thr207Ser + Ser210Glu
	Asp 58Glu + Val 93His + Gly155Pro + Gln200Asn + Thr207Glu
	Thr 64Asn + Asn198Ser + Gln200Glu + Ser206Glu + Thr214Gln
	Gly 59Pro + Gly 61Glu + Ser154Asp + Gln200Asp + Thr214Asn
	Ser126Asp + Pro129Ser + Ala156Asp + Ser210Asp + Leu211Ala
20	Ser103Glu + Ile159Leu + Pro204Glu + Gly205Asn + Ser210Asp
	Ala131Thr + Asn153Glu + Tyr203Asn + Pro204Glu + Ser210Glu
	Gly157Asn + Ser160Glu + Pro204Glu + Gly205Ser + Ser210Glu
	Gly 95Gln + Gly 98Pro + Gly152Glu + Gly157Pro + Val197Asp
	Gln 57Glu + Leu 94Pro + Gly125Ser + Asn153Glu + Leu211Glu
25	Gly 59Ser + Ser104Asp + Ser130Glu + Ser210Glu + Leu211Ser
	Ser104Glu + Ser130Asp + Gly155Asn + Leu211Gly + Gly213Asp
	Ser104Glu + Ser130Glu + Asn198Asp + Gln200Asn + Thr207Gln
	Ser158Glu + Thr202Ser + Thr207Pro + Leu211Thr + Thr214Asp
	Gly 59Glu + Leu 94Val + Ser101Asp + Ala131Glu + Tyr161Met
30	Asp 58Glu + Ser 99Glu + Tyr102Asn + Ala209Asn + Ser210Glu
	Ser101Asp + Phe183Ser + Gln200Asp + Pro204Glu + Thr214Gln
	Gly 61Asp + Gly 98Asn + Ser 99Glu + Ile105Ser + Val197Glu
	Asn 60Ser + Gly125Pro + Ala156Asp + Tyr161Asp + Ser210Asp
	Gly 59Pro + Ser128Glu + Ser154Asp + Thr207Glu + Leu211Ile
35	Ser103Asp + Ala156Gly + Ser182Glu + Asn198Glu + Tyr203His
	Val 93Ala + Gly125Pro + Ser160Glu + Thr207Asp + Leu211Glu
	Ser 97Asp + Gly152Pro + Thr207Glu + Leu211Glu + Gly213Gln
	Gln 57Glu + Ser103Asp + Pro129Glu + Ala181Gly + Thr207Pro
	Ser158Asp + Tyr161Pro + Ser162Asp + Thr207Gln + Ser210Asp

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TABLE 37

Multi-loop Sextuple Mutation Variants	
	Tyr102Cys + Ile105Val + Leu124Ile + Ser154Glu + Asn198Gln
	+ Thr207Gly
45	Ser160Glu + Tyr203Cys + Thr207Pro + Leu211Met + Asn212Gln
	+ Gly213Gln
	Val 93Ser + Ser103Glu + Gly125Ser + Gln185Asn + Ala194Gln
	+ Asn198Gln
	Gly100Ser + Ile105Met + Gly157Pro + Gln200Asn + Thr207Asn
50	+ Leu211Val
	Thr 64Glu + Leu 94Asn + Val197Cys + Thr202Gln + Thr207Gln
	+ Leu211Val

Gly152Pro + Gly157Gln + Ala181Asp + Asn198Gln + Gln200Asn  
 + Thr207Asn  
 Gly 95Gln + Ala 96Asn + Leu124Thr + Asn153Ser + Ser154Glu  
 + Val197His  
 5 Val 93Met + Pro127Ser + Gly155Pro + Ser182Asp + Gln200Ser  
 + Gly213Ser  
 Gly 61Pro + Gly 95Pro + Ser154Glu + Ala181His + Leu211Asn  
 + Gly213Pro  
 Tyr161Thr + Pro195Asn + Gln200Asn + Thr207Gly + Tyr208Leu  
 10 + Leu211Pro  
 Leu 94Pro + Ala 96Ser + Pro127Asp + Ile159Ala + Thr207Pro  
 + Gly213Gln  
 Gly100Asn + Ile105Gln + Gly125Gln + Pro129Gly + Gln185Glu  
 + Thr207Gln  
 15 Gly 98Glu + Pro129Gly + Gly155Pro + Thr207Gly + Ala209His  
 + Thr214Gly  
 Ile105Gln + Gly152Asp + Phe183His + Ala194Gln + Gly205Ser  
 + Asn212Gln  
 Ala194Gln + Asn198Ser + Gln200Asn + Ser206Asp + Leu211His  
 20 + Thr214Gln  
 Ser 99Gln + Pro129Asn + Ala131Pro + Gln185Asn + Thr207Pro  
 + Leu211Val  
 Leu 94Val + Gly 98Gln + Leu124Gly + Val197Glu + Gln200Asn  
 + Thr207Gly  
 25 Gln 57Ser + Gly 98Asn + Tyr102Asp + Tyr161Asn + Gln200Ser  
 + Thr207Gln  
 Asn 60Gln + Gly 61Ser + Tyr102Cys + Gly152Asp + Val199Ser  
 + Pro204Asn  
 Tyr102Thr + Gly155Glu + Ala156Asn + Tyr161Pro + Gln200Asn  
 30 + Leu211Thr  
 Tyr102Glu + Gly157Pro + Phe183His + Asn198Gln + Thr207Pro  
 + Leu211Cys  
 Leu124Cys + Pro127Asn + Tyr208Met + Ser210Asp + Leu211His  
 + Gly213Gln  
 35 Gly 98Ser + Leu124Ala + Ser126Asp + Ala181Asn + Ala209Gly  
 + Gly213Gln  
 Ala 96His + Tyr102Ser + Ile105Ser + Gly157Ser + Ile159Gln  
 + Leu211Asp  
 Thr 64Pro + Ala 96Pro + Ser 97Asp + Pro127Ser + Gly205Asn  
 40 + Leu211Ser  
 Val 93Gly + Gly 98Ser + Tyr102Asn + Val197Met + Thr207Gln  
 + Ser210Asp  
 Asn 60Gln + Ala 96Gln + Asn153Glu + Thr202Gln + Gly205Asn  
 + Leu211Met  
 45 Gly 59Asn + Leu 94Val + Pro127Ser + Ile159Ala + Gln200Asn  
 + Leu211Met  
 Ile105Gln + Gly157Gln + Val193Asn + Val197Asp + Thr207Pro  
 + Tyr208Cys  
 Asn 60Gln + Gly 63Gln + Ile105Glu + Asn198Gln + Leu211Ile  
 50 + Thr214Gly  
 Gly 61Gln + Gly125Glu + Tyr161Gly + Gly205Gln + Thr207Ser  
 + Ala209Gln  
 Gly 98Ser + Ala156Gln + Tyr161Ala + Ser182Glu + Gln200Ser  
 + Thr207Gln

Gly 61Pro + Val 93Ala + Leu124Gly + Ala131Thr + Val197Glu  
 + Asn198Gln  
 Val 93Asp + Ala 96Asn + Val197His + Asn198Ser + Val199Ser  
 + Thr207Gln  
 5 Tyr102Ile + Ile159Asp + Ala181Asn + Gln200Ser + Pro204Gln  
 + Leu211His  
 Gly100Pro + Pro129Ser + Gln200Ser + Thr207Pro + Ala209Glu  
 + Ser210Glu  
 Val197Pro + Asn198Gln + Gln200Glu + Pro204Gly + Thr207Gln  
 10 + Ser210Asp  
 Ala 96Gln + Leu124Met + Ile159Ser + Gln200Glu + Pro204Gly  
 + Ser210Asp  
 Asn 60Gln + Ile159Thr + Asn198Ser + Gln200Glu + Ser210Glu  
 + Leu211Asp  
 15 Gly 61Ser + Val197Gln + Asn198Glu + Gln200Ser + Ser210Asp  
 + Leu211His  
 Gly100Asn + Ala181Pro + Asn198Asp + Thr207Ser + Ser210Glu  
 + Gly213Gln  
 Gly 98Asn + Pro129Ser + Asn198Asp + Gln200Asp + Ser210Glu  
 20 + Leu211Pro  
 Gly 63Ser + Leu 94Gln + Ile159Ala + Gly205Glu + Thr207Glu  
 + Thr214Asn  
 Asp 58Glu + Gly 61Glu + Gly 98Asn + Asn153Ser + Tyr161Asn  
 + Leu211Cys  
 25 Leu124Ser + Ile159Thr + Asn198Glu + Gln200Glu + Pro204Gly  
 + Thr207Gln  
 Gly 61Gln + Gly 95Asn + Ala194Gly + Asn198Asp + Gln200Asp  
 + Leu211Val  
 Gln 57Ser + Tyr102Gln + Asn198Glu + Ala209Glu + Ser210Glu  
 30 + Leu211Ser  
 Gly 59Gln + Gly125Pro + Ser154Glu + Gly155Asp + Tyr161Ala  
 + Ser182Asp  
 Gln 57Glu + Gly 59Asp + Thr 64Gln + Tyr206Pro + Ala209Gln  
 + Leu211Asn  
 35 Asn 60Asp + Gly 61Gln + Gly 95Glu + Gly 98Glu + Asn153Ser  
 + Ile159Leu  
 Ile105Thr + Gly152Ser + Ser158Asp + Ser160Asp + Thr207Gly  
 + Leu211Ala  
 Gly 61Gln + Thr 64Asn + Asn198Glu + Ser210Asp + Leu211Asp  
 40 + Gly213Asp  
 Leu 94Pro + Ala 96Gly + Thr207Ser + Ser210Asp + Leu211Cys  
 + Asn212Glu  
 Gly 98Glu + Gly100Asp + Ala181Pro + Tyr203Val + Pro204Asn  
 + Thr207Ser  
 45 Asp 58Glu + Gly157Ser + Gln185Asn + Val197Gln + Pro204Glu  
 + Asn212Gln  
 Gly 61Asn + Leu124Thr + Ser128Glu + Ser160Glu + Ala181Asn  
 + Thr207Gln  
 Ile105Leu + Leu124Ser + Gly125Asp + Gly152Glu + Asn153Glu  
 50 + Gly213Glu  
 Leu124Gln + Asn198Glu + Gln200Asp + Thr207Gly + Tyr208Glu  
 + Leu211Asp  
 Leu 94Ile + Gly152Ser + Gly155Glu + Ser158Asp + Ala181Gly  
 + Val197His

Asp 58Glu + Tyr161Gly + Val197Ala + Asn198Gln + Pro204Glu  
 + Ser206Asp  
 Gln 57Ser + Ile159Ser + Asn198Asp + Gln200Asn + Tyr208Asn  
 + Gly213Glu  
 5 Gly152Ser + Phe183Ala + Asn198Glu + Val199Ser + Leu211Cys  
 + Gly213Glu  
 Leu 94Ile + Ser154Asp + Ile159Glu + Asn198Ser + Gln200Asn  
 + Leu211Met  
 Ala 96Pro + Ser154Asp + Ala156His + Gly157Asn + Ser158Glu  
 10 + Pro204Ser  
 Gln 57Asp + Gly 59Pro + Asn 60Asp + Gly 98Pro + Thr207Ser  
 + Ala209Gln  
 Thr 64Ser + Gly152Gln + Ala181His + Gln200Glu + Leu211His  
 + Asn212Glu  
 15 Gln 57Asn + Gly 98Gln + Gln200Asp + Leu211Cys + Asn212Asp  
 + Gly213Ser  
 Ala 96Asn + Gly100Asp + Leu124Asp + Val197Gly + Thr207Pro  
 + Gly213Ser  
 Ala 96Ser + Ser154Asp + Phe183Gln + Thr207Ser + Leu211Thr  
 20 + Gly213Asp  
 Gly 95Asp + Gly 98Pro + Ser 99Asp + Gly100Ser + Tyr102Glu  
 + Ser103Glu  
 Gln 57Asp + Asp 58Glu + Thr 64Asp + Ser 97Glu + Gln200Ser  
 + Tyr208Thr  
 25 Gly125Asp + Ser130Asp + Asn153Glu + Ala156Thr + Ser160Glu  
 + Asn198Ser  
 Gly 59Asn + Leu124Thr + Ile159Met + Ala209Asp + Leu211Cys  
 + Asn212Asp  
 Gly 95Gln + Gly100Asn + Ile105Met + Ala181Gln + Gln200Glu  
 30 + Thr207Glu  
 Ile105Met + Ser126Asp + Asn153Glu + Gly205Gln + Thr207Pro  
 + Leu211Met  
 Pro129Gly + Ala131His + Val197Thr + Thr207Asn + Ser210Glu  
 + Gly213Glu  
 35 Gly 98Asp + Gly152Glu + Asn153Glu + Ser154Asp + Val197Ser  
 + Gln200Asn  
 Asp 58Glu + Gly 61Pro + Val 93Gly + Ser 97Glu + Pro129Asn  
 + Tyr161Ile  
 Ser130Asp + Gly152Ser + Val197Gly + Asn198Glu + Ser210Glu  
 40 + Leu211Asp  
 Tyr102Val + Gly155Asn + Ser182Asp + Val197Asp + Gln200Asp  
 + Ser210Asp  
 Thr 64Gly + Ser128Glu + Gln200Glu + Thr207Ser + Ser210Asp  
 + Leu211Asp  
 45 Gln 57Glu + Tyr102Ser + Thr207Pro + Ser210Asp + Leu211Asp  
 + Asn212Asp  
 Pro127Asn + Gly152Glu + Ile159Asn + Asn198Asp + Gln200Asn  
 + Gly213Glu  
 Asn153Ser + Gly155Ser + Ile159Thr + Asn198Asp + Ser210Glu  
 50 + Thr214Asp  
 Val 93Thr + Ser154Asp + Ser158Glu + Ser182Glu + Asn198Ser  
 + Thr207Ser  
 Ser101Asp + Gly155Pro + Asn198Glu + Gln200Glu + Ala209Asn  
 + Ser210Asp

Ser 99Glu + Ser126Asp + Pro127Asp + Ile159Ser + Gln200Ser  
 + Leu211Met  
 Pro129Asn + Asn153Asp + Ser156Asp + Gln200Ser + Pro204Ser  
 + Gly205Ser  
 5 Val197Cys + Gly205Asp + Thr207Asp + Ser210Asp + Leu211Ile  
 + Asn212Ser  
 Gly 61Asn + Asn198Asp + Gln200Glu + Thr207Glu + Tyr208Cys  
 + Leu211His  
 Asn 60Asp + Pro127Gln + Asn153Glu + Ser154Glu + Ala156Glu  
 10 + Thr207Pro  
 Ser 99Glu + Asn153Asp + Ser154Asp + Ala156Glu + Gln185Asn  
 + Pro195Ser  
 Thr 64Asn + Ala156Asp + Val197Asp + Gln200Glu + Leu211Pro  
 + Thr214Glu  
 15 Ser126Asp + Pro127Gln + Ser128Asp + Ser160Asp + Gln200Glu  
 + Thr214Ser  
 Asn153Asp + Gln185Glu + Asn198Ser + Gln200Glu + Leu211Cys  
 + Gly213Asp  
 Ile105Gln + Leu124Glu + Gly125Glu + Ser160Asp + Ala194Gln  
 20 + Pro204Glu  
 Asp 58Glu + Leu 94Pro + Ser154Glu + Gly155Asp + Ser182Asp  
 + Asn198Gln  
 Ser 99Glu + Val197Glu + Thr207Gly + Ala209Asn + Ser210Glu  
 + Leu211Asp  
 25 Gln 57Asn + Gly125Glu + Asn153Glu + Gln200Asn + Leu211Ser  
 + Asn212Asp  
 Leu 94Ser + Gly125Gln + Ser126Glu + Val197Asp + Ser210Asp  
 + Asn212Glu  
 Thr 64Glu + Ile159His + Asn198Glu + Gln200Asp + Leu211Cys  
 30 + Thr214Gly  
 Gly 98Asp + Gly152Glu + Gly155Glu + Ala156Asp + Tyr208Met  
 + Asn212Ser  
 Gly 98Pro + Ser104Glu + Asn198Glu + Gln200Asp + Gly205Gln  
 + Asn212Asp  
 35 Asp 58Glu + Thr 64Glu + Ala 96Ser + Tyr102His + Gln200Glu  
 + Leu211Gly  
 Leu 94Ala + Gly100Gln + Phe183Asp + Val193Gln + Val197Asp  
 + Gln200Glu  
 Asn 60Gln + Thr 64Gly + Gly125Glu + Ser154Glu + Gly157Asp  
 40 + Thr207Gly  
 Val 93Met + Gly100Asp + Ser126Glu + Pro129Asp + Ala181Asn  
 + Thr214Ser  
 Gly125Asn + Ser182Glu + Phe183Tyr + Gln185Asp + Asn198Asp  
 + Asn212Glu  
 45 Leu 94Gln + Gly125Asp + Ser128Glu + Pro129Glu + Asn198Gln  
 + Ser210Glu  
 Gly 59Gln + Thr 64Asp + Asn198Gln + Gln200Asn + Ser210Asp  
 + Asn212Asp  
 Gly 59Gln + Gly125Glu + Asn198Asp + Gln200Asp + Leu211Ile  
 50 + Gly213Asp  
 Thr 64Asn + Gly125Glu + Asn153Asp + Gln185Glu + Thr207Ser  
 + Leu211Asp  
 Asp 58Glu + Gly 95Glu + Gly 98Glu + Gln185Asn + Pro204Ser  
 + Ser210Asp

Gln 57Glu + Gly 59Glu + Thr 64Asp + Gly152Ser + Ala156Glu  
 + Leu211Ser  
 Asn 60Ser + Ala156Gln + Asn198Glu + Gly205Gln + Thr207Asp  
 + Leu211Asp  
 5 Leu 94Ala + Ser101Asp + Ser154Glu + Ser158Asp + Ser160Asp  
 + Leu211Thr  
 Asp 58Glu + Gln200Glu + Thr207Glu + Ser210Glu + Leu211Thr  
 + Gly213Pro  
 Gly 61Gln + Gly152Asp + Gln200Glu + Ser210Glu + Leu211Gly  
 10 + Thr214Asp  
 Asn 60Glu + Tyr102Ile + Gln200Glu + Thr207Asp + Ser210Asp  
 + Asn212Ser  
 Gly 59Ser + Val 93Asp + Ile159Asp + Ser160Glu + Thr207Asn  
 + Gly213Ser  
 15 Thr 64Asn + Ser104Asp + Ile105Cys + Ala156Gly + Ser210Asp  
 + Leu211Asp  
 Gln 57Asn + Gly 98Asp + Gly100Gln + Ser206Glu + Thr207Glu  
 + Tyr208Met  
 Gln 57Asn + Ser158Asp + Ile159Glu + Phe183Met + Asn198Asp  
 20 + Thr207Asn  
 Gly 95Asp + Ala 96Gly + Ala131Gln + Thr207Glu + Tyr208Glu  
 + Leu211Thr  
 Ser103Asp + Ser104Asp + Gln200Asn + Leu211Asn + Asn212Asp  
 + Gly213Asp  
 25 Gly 61Gln + Gly 98Glu + Gly155Asp + Phe183Asp + Thr207Gln  
 + Leu211Pro  
 Leu 94Ser + Gly 95Ser + Ser 97Glu + Gly 98Glu + Ala131Glu  
 + Asn212Ser  
 Thr 64Asn + Tyr102Ile + Ser103Asp + Ser104Asp + Ala181Asn  
 30 + Tyr208Asp  
 Thr 64Gly + Tyr102Asp + Gln200Glu + Thr207Asn + Tyr208Gln  
 + Ser210Glu  
 Ser 99Glu + Tyr102His + Ile159Ala + Gln200Asp + Thr207Gln  
 + Ser210Glu  
 35 Thr 64Glu + Leu124Cys + Val197Glu + Gln200Asn + Ser210Asp  
 + Leu211Gly  
 Thr 64Gly + Ser154Asp + Ala181Asn + Gln200Glu + Thr207Glu  
 + Ser210Asp  
 Tyr102Ala + Ala181Asn + Ser182Glu + Gln200Asp + Thr207Asp  
 40 + Ser210Asp  
 Gly125Pro + Gly152Asn + Ser158Glu + Ile159Glu + Asn198Asp  
 + Ser210Glu  
 Gln 57Ser + Asn 60Ser + Val 93Gln + Leu 94Gly + Gln200Glu  
 + Gly213Asp  
 45 Gly 61Glu + Thr 64Ser + Val 93Asp + Asn198Ser + Pro204Glu  
 + Tyr208Gly  
 Ser101Asp + Ser103Glu + Leu124Met + Gly155Ser + Ser210Asp  
 + Leu211Gln  
 Ser 99Glu + Pro127Asp + Pro129Ser + Ser154Glu + Ser158Glu  
 50 + Thr207Ser  
 Gly 61Glu + Ser101Glu + Ser104Glu + Ile159Leu + Gly196Asn  
 + Asn198Gln  
 Gly 59Asp + Asn 60Glu + Ser 99Glu + Val197Thr + Thr207Asp  
 + Gly213Gln

Gly 61Asn + Ala 96Glu + Gly 98Glu + Leu124Gln + Pro127Ser  
 + Gly152Glu  
 Gly125Ser + Phe183Asp + Gln185Glu + Ala209Glu + Ser210Asp  
 + Leu211His  
 5 Ser130Asp + Ile159Gly + Asn198Glu + Thr207Gln + Ser210Asp  
 + Leu211Met  
 Pro127Gly + Ser130Asp + Tyr161Thr + Asn198Glu + Ser210Glu  
 + Thr214Gln  
 Gly 98Pro + Tyr102Glu + Phe183Met + Asn198Asp + Gln200Asn  
 10 + Ser210Asp  
 Ser 99Glu + Pro127Ser + Gly155Pro + Val197Cys + Asn198Glu  
 + Leu211Glu  
 Leu 94Ser + Ser101Glu + Ile105Glu + Pro195Gln + Val197Glu  
 + Asn198Glu  
 15 Pro127Glu + Asn198Ser + Pro204Glu + Gly205Ser + Ser206Asp  
 + Thr207Pro  
 Gln 57Ser + Asn 60Asp + Gln185Asn + Asn198Glu + Leu211Thr  
 + Asn212Asp  
 Asp 58Glu + Asn 60Asp + Tyr102Ala + Ile159Ser + Ser160Glu  
 20 + Gly205Pro  
 Ala 96Gly + Ala181Asp + Asn198Asp + Thr207Glu + Ala209His  
 + Ser210Glu  
 Gln 57Asn + Asn 60Glu + Gly 95Glu + Gly100Glu + Gly125Ser  
 + Ser210Glu  
 25 Asp 58Glu + Phe183Ser + Pro204Glu + Thr207Ser + Ser210Glu  
 + Asn212Glu  
 Leu 94Asp + Pro129Glu + Ala156Ser + Tyr161Asp + Gln185Ser  
 + Asn212Ser  
 Gly 95Glu + Ser 99Asp + Ala156His + Val197Glu + Asn198Gln  
 30 + Thr207Asn  
 Thr 64Glu + Pro127Asn + Ala156Glu + Ala209Asp + Ser210Asp  
 + Leu211Asn  
 Gly 59Ser + Gly 63Pro + Pro129Ser + Gly157Glu + Gln185Asp  
 + Ser210Glu  
 35 Gln 57Ser + Gly 59Asp + Leu 94Glu + Gly125Asp + Ser160Glu  
 + Gln185Asn  
 Ile105Gln + Ser126Glu + Ser126Asp + Leu211Glu + Gly213Asp  
 + Thr214Gly  
 Pro127Ser + Ser182Glu + Gln185Glu + Asn198Asp + Thr207Pro  
 40 + Ser210Glu  
 Ala 96Asn + Ala131Pro + Ala181Asp + Thr207Asp + Ser210Asp  
 + Leu211Asp  
 Asp 58Glu + Gly 59Pro + Ser126Asp + Gly152Asp + Ser158Glu  
 + Leu211Ile  
 45 Gly155Asp + Ser182Asp + Val197Gly + Gln200Asp + Thr202Asn  
 + Leu211Glu  
 Ser126Asp + Ile159Ser + Asn198Ser + Gln200Ser + Pro204Glu  
 + Thr207Asp  
 Asn 60Ser + Gly 63Asn + Gln185Glu + Pro195Ser + Asn198Asp  
 50 + Gln200Glu  
 Gly 59Ser + Ile105Asp + Ala181Asn + Asn198Asp + Gln200Asp  
 + Leu211Thr  
 Leu 94Glu + Ser 99Asp + Gly100Gln + Ser103Asp + Gln200Glu  
 + Tyr208Met



Asp 58Glu + Leu 94Asp + Ser 99Glu + Gly100Pro + Gly157Asp  
 + Tyr203Val  
 Asp 58Glu + Thr 64Gln + Gly 98Glu + Ser 99Asp + Pro129Glu  
 + Gly155Ser  
 5 Val 93Gln + Ser101Asp + Ile105Asp + Asn198Asp + Gly205Ser  
 + Leu211Asn  
 Ser 99Glu + Ile105Ala + Ser210Glu + Leu211His + Asn212Glu  
 + Thr214Asp  
 Val 93Ala + Leu124His + Ala131Gly + Gly157Asp + Val197Asp  
 10 + Gly213Asp  
 Ile105His + Gly152Ser + Ala156Asn + Ser158Glu + Val197Asp  
 + Gly213Asp  
 Gly 61Asp + Leu 94Thr + Val197Met + Asn198Gln + Gln200Glu  
 + Gly205Glu  
 15 Tyr102Pro + Ser128Asp + Ser130Glu + Ala131Thr + Gln200Asp  
 + Leu211Asp  
 Gly 98Ser + Ser126Asp + Ser128Glu + Gly152Gln + Ser158Asp  
 + Gly205Glu  
 Ser130Asp + Phe183Ile + Thr207Gly + Tyr208Asp + Ser210Glu  
 20 + Asn212Asp  
 Asp 58Glu + Leu 94Thr + Gly100Gln + Tyr102His + Ser206Asp  
 + Thr214Pro  
 Asp 58Glu + Thr 64Glu + Ser126Glu + Pro129Ser + Ala181Gly  
 + Asn198Gln  
 25 Asn 60Ser + Leu 94Asp + Ala 96Glu + Ala181Glu + Gln185Asn  
 + Thr207Gly  
 Pro127Asp + Pro129Glu + Ala181Gln + Pro195Ser + Ser206Asp  
 + Thr214Gln  
 Gly152Gln + Ala156Asp + Thr207Asp + Ala209Glu + Leu211Pro  
 30 + Gly213Pro  
 Ser103Glu + Gly157Ser + Ser158Glu + Ser160Glu + Tyr161His  
 + Pro204Ser  
 Leu 94Asp + Gly 95Gln + Gly 98Asp + Ser126Glu + Ser154Asp  
 + Thr207Ser  
 35 Ser 97Glu + Ile105Glu + Leu124Asp + Phe183Gly + Thr207Gly  
 + Leu211Gln  
 Gly100Glu + Pro129Ser + Ala131Ser + Tyr161Val + Ser210Asp  
 + Asn212Glu  
 Gly 59Ser + Ser101Glu + Ile105Gln + Ala131His + Ser210Glu  
 40 + Asn212Glu  
 Gln 57Asn + Pro127Gln + Ala156Asp + Ile159Asp + Pro195Gly  
 + Ser206Asp  
 Val 93His + Gly100Glu + Ser126Asp + Ser128Glu + Gln185Asp  
 + Pro204Gly  
 45 Gly 95Ser + Ser 99Asp + Ser101Asp + Val197Asn + Leu211Met  
 + Gly213Glu  
 Gln 57Ser + Gly 61Glu + Ala 96Glu + Asn153Asp + Gly213Pro  
 + Thr214Glu  
 Gln 57Ser + Ala156Asp + Ser160Asp + Val197Gly + Leu211Asp  
 50 + Gly213Asp  
 Gly 98Pro + Ser104Asp + Ala181Glu + Ser182Glu + Asn198Glu  
 + Asn212Gln  
 Ile105Glu + Leu124Thr + Val197Ser + Pro204Glu + Thr207Asp  
 + Ser210Asp

Asn 60Ser + Gly 61Pro + Ser160Asp + Gln185Asp + Gln200Asn  
 + Ser210Glu  
 Gly 61Ser + Leu 94Asp + Ser104Asp + Leu124Asn + Asn198Ser  
 + Gly213Asp  
 5 Asn 60Glu + Val 93Met + Gly 95Asp + Gly 98Asn + Asn198Glu  
 + Leu211Cys  
 Gly 95Ser + Gly 98Asp + Ser 99Glu + Ser126Glu + Ser182Glu  
 + Thr207Gly  
 Asp 58Glu + Gly 98Asp + Leu124Cys + Gln200Asp + Ser210Asp  
 10 + Asn212Ser  
 Leu 94Glu + Ser 97Asp + Ser158Glu + Val197Thr + Tyr208Ser  
 + Leu211Ser  
 Ser101Glu + Ile105Glu + Ser154Glu + Gly157Glu + Asn198Ser  
 + Leu211Ala  
 15 Gly 63Ser + Gly 98Glu + Gly100Pro + Gly152Asp + Gln200Asn  
 + Gly213Asp  
 Leu 94Glu + Ser101Asp + Phe183Ala + Gln200Asn + Ser206Glu  
 + Leu211Met  
 Gln 57Glu + Asp 58Glu + Leu124Pro + Asn198Ser + Ser206Asp  
 20 + Gly213Asp  
 Gly125Glu + Ala131His + Gly205Asp + Ala209Glu + Ser210Glu  
 + Thr214Ser  
 Gly 59Ser + Gly100Glu + Ser103Asp + Asn198Ser + Tyr208Leu  
 + Ser210Glu  
 25 Asp 58Glu + Asn 60Asp + Gly155Asp + Val197Asn + Thr207Ser  
 + Thr214Asp  
 Ser 99Asp + Ser128Glu + Pro129Asp + Gly152Glu + Gln200Asn  
 + Thr207Asn  
 Pro127Gln + Asn153Glu + Ser182Asp + Val197Cys + Ser210Glu  
 30 + Leu211Pro  
 Ser104Glu + Leu124Pro + Asn153Glu + Ala156Thr + Ile159Leu  
 + Ser182Glu  
 Thr 64Asn + Tyr102Cys + Ser128Asp + Ser158Asp + Phe183Glu  
 + Asn198Ser  
 35 Ala 96Gln + Tyr161Asn + Gln185Asn + Val197Glu + Gly205Glu  
 + Ser210Asp  
 Gly 98Ser + Ser101Glu + Pro127Gly + Gln200Asp + Leu211Asp  
 + Thr214Glu  
 Gly 59Gln + Thr 64Glu + Gly 95Ser + Gly155Glu + Ser158Asp  
 40 + Val197Cys  
 Tyr102Val + Ser103Glu + Asn198Asp + Gln200Glu + Thr207Glu  
 + Ala209Asn  
 Asn 60Glu + Gly 98Gln + Asn153Glu + Ser210Asp + Leu211Ala  
 + Gly213Glu  
 45 Asp 58Glu + Gly 61Glu + Ser101Glu + Leu124Asn + Pro129Gly  
 + Ser130Asp  
 Ser126Glu + Ser130Asp + Ala131Thr + Gln200Asn + Thr202Gln  
 + Pro204Asp  
 Gly 63Gln + Ser 97Glu + Ser126Asp + Ser130Asp + Asn198Ser  
 50 + Gln200Ser  
 Val 93Gln + Leu 94Cys + Ser 97Glu + Ser101Glu + Asn212Gln  
 + Gly213Ser  
 Asp 58Glu + Gly 95Glu + Ser154Asp + Ser158Asp + Thr207Pro  
 + Tyr208Val

Asn 60Gln + Thr 64Ser + Pro129Asn + Pro204Asp + Ser210Asp  
 + Leu211Ile  
 Ser 99Asp + Ile159Gln + Val197Gly + Asn198Glu + Leu211Val  
 + Gly213Asp  
 5 Ser 97Glu + Gly100Glu + Gly152Glu + Gly155Gln + Ala181Ser  
 + Ala209His  
 Asp 58Glu + Ala 96Glu + Ser126Glu + Asn153Asp + Phe183Leu  
 + Gly205Asn  
 Gly 98Asp + Pro127Glu + Gly152Pro + Ser158Glu + Gly205Pro  
 10 + Leu211Gln  
 Ser154Glu + Gly157Asp + Ile159Asn + Asn198Glu + Gln200Asn  
 + Tyr203Asn  
 Gly 63Ser + Phe183Glu + Thr207Glu + Ser210Glu + Leu211Pro  
 + Thr214Asp  
 15 Gly100Glu + Asn153Ser + Ser154Asp + Ser160Glu + Asn198Ser  
 + Gly213Glu  
 Gln 57Glu + Ala 96His + Gly 98Pro + Ser160Asp + Pro204Asp  
 + Tyr208Gly  
 Gly 59Asp + Gly 95Pro + Ala194Gln + Asn198Asp + Gln200Asp  
 20 + Thr214Asp  
 Gly 95Glu + Val197Glu + Asn198Gln + Gln200Glu + Thr207Gln  
 + Leu211Ala  
 Gly 98Asp + Ser128Asp + Gly155Pro + Ser158Asp + Gln185Glu  
 + Gln200Ser  
 25 Gly 98Glu + Leu124Asp + Gly125Asn + Ile159Gly + Val197Asp  
 + Leu211Ala  
 Thr 64Asp + Gly152Asn + Ser160Glu + Gln185Asn + Thr207Asp  
 + Ser210Asp  
 Thr 64Asp + Gly155Asp + Ala156Ser + Val197Ser + Asn198Glu  
 30 + Leu211Asp  
 Gln 57Asn + Thr 64Pro + Ile105Glu + Ser126Glu + Ser160Asp  
 + Ser206Glu  
 Gly 98Asn + Gly157Asp + Phe183Glu + Val197Asn + Ser206Asp  
 + Thr214Glu  
 35 Ser 97Asp + Ser126Glu + Ser128Asp + Pro129Asn + Ala156Asp  
 + Leu211Gln  
 Gln 57Glu + Leu124Pro + Phe183Asn + Val197Glu + Gly205Glu  
 + Gly213Glu  
 Ser126Asp + Ser158Glu + Gln200Asp + Thr207Pro + Leu211Glu  
 40 + Asn212Ser  
 Gly 98Ser + Gly100Pro + Pro129Glu + Gly157Glu + Ser158Glu  
 + Asn198Asp  
 Gly 61Asp + Gln185Asp + Asn198Gln + Gln200Asp + Thr207Asp  
 + Leu211Ser  
 45 Asp 58Glu + Asn198Gln + Gln200Glu + Thr207Glu + Leu211Ala  
 + Asn212Glu  
 Gly 59Asp + Ser 97Glu + Pro129Asn + Gly152Pro + Ser182Glu  
 + Thr214Asp  
 Gly 59Glu + Thr 64Glu + Pro127Gln + Pro129Asn + Ala131Gly  
 50 + Asn153Glu  
 Pro127Glu + Ser130Asp + Ser154Glu + Ile159Cys + Ser210Glu  
 + Leu211Ser  
 Asp 58Glu + Val 93Asn + Ser 97Asp + Gly100Glu + Gly152Pro  
 + Asn198Glu

Gly125Glu + Gly155Asp + Gly157Asn + Gln200Glu + Gly205Asn  
 + Ser210Asp  
 Ala 96Ser + Ser103Glu + Leu124Asp + Gln200Glu + Ser210Glu  
 + Leu211Cys  
 5 Ser101Asp + Pro129Asp + Gly155Asn + Ala181Glu + Ser182Asp  
 + Gly205Pro  
 Asp 58Glu + Gly 61Glu + Ala 96His + Asn153Asp + Val197Gln  
 + Gln200Glu  
 Asn 60Asp + Ser 99Glu + Gly155Pro + Ala181Glu + Val197Asn  
 10 + Asn212Asp  
 Asn 60Gln + Gly 98Asn + Ser130Asp + Ala181Asp + Ala209Asp  
 + Asn212Asp  
 Gly 61Asp + Ala131Asp + Val197Ala + Asn198Asp + Gln200Asp  
 + Thr214Asn  
 15 Thr 64Asp + Ser 99Asp + Ser101Glu + Ala181Gln + Gln200Ser  
 + Ser210Glu  
 Leu124Ser + Tyr161Glu + Ser182Asp + Val197Glu + Thr207Gln  
 + Leu211Asn  
 Gly 95Glu + Gly 98Asp + Ser103Asp + Ala181Gln + Thr207Gln  
 20 + Asn212Asp  
 Asn 60Glu + Ser 97Glu + Ser101Glu + Leu124Ala + Pro127Glu  
 + Val197Gln  
 Val 93Asp + Ala 96Glu + Ala131Asp + Gly152Gln + Gly196Ser  
 + Val197His  
 25 Val 93Gly + Ala131Asp + Ile159Gly + Ser182Glu + Asn198Gln  
 + Gly213Asp  
 Ser130Asp + Ser182Asp + Gln185Ser + Val197Asn + Asn198Glu  
 + Ser210Asp  
 Ile159Thr + Ser182Asp + Gln200Asp + Gly205Asp + Thr207Asn  
 30 + Asn212Glu  
 Val 93Thr + Ser104Glu + Pro127Ser + Ser128Asp + Ser130Glu  
 + Asn153Glu  
 Asn 60Glu + Leu 94Asp + Gln200Ser + Gly205Asp + Ser210Asp  
 + Leu211Gln  
 35 Gly 95Asp + Ser101Asp + Ser130Glu + Asn198Asp + Gln200Ser  
 + Leu211Ala  
 Ser104Asp + Ser154Glu + Thr207Asn + Ala209Asp + Ser210Glu  
 + Leu211Asn  
 Asp 58Glu + Gly 59Glu + Ile105Leu + Ser126Asp + Phe183Asn  
 40 + Ser210Asp  
 Gly 59Glu + Asn 60Asp + Gly 95Asn + Pro129Glu + Gly152Ser  
 + Gln185Glu  
 Gly 59Asp + Ser160Glu + Gly196Ser + Gly205Ser + Ser210Asp  
 + Leu211Asp  
 45 Asn 60Glu + Ser 99Asp + Gly157Glu + Phe183Leu + Val197Asn  
 + Thr207Gln  
 Ser 99Glu + Asn198Asp + Ser206Glu + Thr207Asp + Tyr208Asn  
 + Leu211His  
 Gly 98Glu + Ser 99Asp + Ala131Pro + Asn153Glu + Tyr161Pro  
 50 + Tyr208Glu  
 Ser 97Glu + Gly 98Glu + Ser182Asp + Thr207Gln + Ser210Asp  
 + Gly213Ser  
 Ser 97Asp + Gly 98Asp + Ile105Val + Pro129Gly + Gln200Asp  
 + Gly205Glu

Asp 58Glu + Ser103Asp + Ser104Asp + Phe183Gly + Val197Pro  
 + Asn198Asp  
 Asn 60Ser + Ser 99Glu + Gly100Asp + Pro129Gly + Gly205Asp  
 + Ser210Glu  
 5 Ser 99Glu + Ser154Asp + Tyr161Cys + Val197Ser + Gln200Asp  
 + Ser210Asp  
 Ser101Glu + Gly155Asp + Gln185Ser + Asn198Gln + Gln200Asp  
 + Ser210Glu  
 Gly 59Asp + Tyr102Asn + Ser158Asp + Gln200Asp + Tyr206Val  
 10 + Ser210Glu  
 Gly 59Asp + Ala156Gln + Ile159Asp + Phe183Gly + Gln200Asp  
 + Ser210Asp  
 Pro127Ser + Ser160Asp + Ser182Asp + Val197Gln + Gln200Glu  
 + Ser210Asp  
 15 Gln 57Asn + Val 93Asp + Pro127Glu + Asn198Gln + Gln200Glu  
 + Ser210Glu  
 Leu 94Glu + Pro129Gln + Ser154Asp + Val197His + Gln200Asp  
 + Ser210Glu  
 Ala 96Ser + Gly 98Gln + Ser126Asp + Ser154Asp + Val197Thr  
 20 + Thr207Asp  
 Gln 57Ser + Ser104Asp + Gly125Asp + Ser160Glu + Gly205Pro  
 + Ser210Glu  
 Leu 94Gln + Ser 99Asp + Gly155Pro + Val193Asn + Gln200Asp  
 + Thr207Asp  
 25 Gly 95Glu + Gln200Glu + Thr202Ser + Thr207Asp + Leu211Gln  
 + Thr214Gln  
 Thr 64Gln + Ala 96Thr + Ser104Asp + Gln200Asp + Thr207Asp  
 + Thr214Pro  
 Thr 64Glu + Tyr102Cys + Ala156Ser + Phe183Glu + Pro195Gln  
 30 + Thr207Glu  
 Gly 98Glu + Val197His + Thr207Gln + Ala209Ser + Ser210Asp  
 + Gly213Asp  
 Gly 95Ser + Gly157Asp + Phe183Glu + Thr207Asp + Ala209Gln  
 + Ser210Asp  
 35 Asp 58Glu + Val 93Asp + Ser128Glu + Ala131Glu + Gly196Gln  
 + Pro204Asn  
 Gln 57Ser + Pro127Glu + Gln185Asn + Asn198Asp + Gln200Glu  
 + Ser206Glu  
 Val 93Glu + Gly152Asn + Thr207Glu + Ser210Asp + Leu211Ser  
 40 + Gly213Glu  
 Thr 64Gly + Ser 99Glu + Ser103Glu + Pro127Asn + Gly152Glu  
 + Ser158Glu  
 Gly157Asn + Ser158Glu + Asn198Asp + Ser206Asp + Thr207Gln  
 + Ser210Asp  
 45 Ala 96Asp + Ser126Glu + Ser160Glu + Tyr161Leu + Ser210Glu  
 + Gly213Ser  
 Asp 58Glu + Ala 96Ser + Ser101Asp + Ser104Glu + Ala209Gln  
 + Ser210Asp  
 Gly 61Asp + Thr 64Gln + Val 93Cys + Ile105Ser + Leu211Val  
 50 + Asn212Glu  
 Gly 61Glu + Phe183Ser + Tyr203Thr + Thr207Asn + Tyr208Gly  
 + Asn212Glu  
 Ser 97Glu + Ile105Cys + Pro127Ser + Ser160Asp + Asn198Glu  
 + Ser210Asp

Leu 94Asp + Ser130Glu + Asn198Asp + Pro204Asn + Thr207Gln  
 + Ser210Asp  
 Ile105Asp + Leu124Pro + Ile159Leu + Asn198Glu + Ser206Asp  
 + Leu211Glu  
 5 Tyr102Gly + Ala131Ser + Ser182Glu + Phe183Pro + Thr207Gln  
 + Ser210Asp  
 Asn 60Gln + Gly 61Glu + Ser 97Asp + Gly125Glu + Tyr208Pro  
 + Ser210Asp  
 Gly 98Gln + Ser160Glu + Ala181Thr + Asn198Asp + Ser206Asp  
 10 + Asn212Glu  
 Leu124Ala + Ser128Glu + Asn153Gln + Gly205Asp + Thr207Glu  
 + Asn212Glu  
 Gly 59Glu + Gly100Pro + Ser103Glu + Ile105Glu + Val197Gly  
 + Ser210Glu  
 15 Asn 60Glu + Ser 97Glu + Pro127Asp + Val193Met + Thr207Asp  
 + Ala209Asn  
 Thr 64Gln + Ala 96Asn + Ser101Glu + Ser130Asp + Asn198Ser  
 + Leu211Asp  
 Gly 61Pro + Leu 94Glu + Ser103Asp + Ile159Pro + Leu211Asn  
 20 + Asn212Glu  
 Val 93Cys + Leu 94Asp + Gly100Ser + Ser103Glu + Ser206Asp  
 + Tyr208Gly  
 Ser130Asp + Gly152Glu + Ser154Glu + Gly157Ser + Ala181Pro  
 + Asn198Glu  
 25 Gly 59Glu + Ser130Asp + Ser154Asp + Gln185Asp + Thr207Gly  
 + Leu211Pro  
 Gln 57Glu + Leu 94Ala + Ser101Asp + Ser126Glu + Ala131Gln  
 + Ser154Asp  
 Ser103Asp + Ile105Met + Gly125Asn + Ser160Asp + Val197Ser  
 30 + Thr214Asp  
 Asp 58Glu + Val 93Asp + Gly125Ser + Gly152Ser + Val193His  
 + Gln200Glu  
 Asp 58Glu + Gly 98Asp + Leu124Ser + Gly125Glu + Tyr161Met  
 + Gln200Glu  
 35 Gly 61Asp + Leu 94Ile + Asn153Asp + Ser158Asp + Val197Ala  
 + Ser210Glu  
 Asn 60Ser + Val 93Ser + Ser103Glu + Ser160Glu + Asn198Asp  
 + Gln200Asp  
 Asn 60Gln + Ser 97Asp + Ser128Asp + Asn198Glu + Gln200Asp  
 40 + Ala209Gly  
 Val 93Ser + Tyr102Asn + Ser104Asp + Gly152Asp + Asn198Glu  
 + Gln200Glu  
 Gly 59Asp + Ile105Met + Ser158Glu + Ile159Ser + Asn198Glu  
 + Gln200Asp  
 45 Gly 59Glu + Ser101Glu + Ile159Cys + Asn198Glu + Val199Gln  
 + Gln200Asp  
 Gly 61Asp + Pro127Asp + Gln185Asp + Asn198Ser + Gln200Ser  
 + Ser210Asp  
 Gly100Glu + Ser104Glu + Ser182Glu + Thr207Gly + Tyr208Met  
 50 + Ser210Glu  
 Thr 64Gly + Gly157Asp + Ser182Glu + Val197Gln + Asn198Ser  
 + Ser210Asp  
 Ser128Glu + Ser130Asp + Tyr161Val + Asn198Glu + Ser206Glu  
 + Gly213Asn

Gly 59Gln + Thr 64Asp + Ser 99Glu + Asn198Asp + Pro204Glu  
 + Thr207Asn  
 Gly 95Glu + Ser126Asp + Gln185Ser + Val197Cys + Gln200Glu  
 + Leu211Glu  
 5 Gly 98Pro + Ser 99Glu + Ser158Asp + Gln200Asp + Ala209Ser  
 + Leu211Asp  
 Ser101Glu + Pro129Glu + Asn153Ser + Ser160Asp + Thr207Glu  
 + Leu211Cys  
 10 Asn 60Glu + Gly 98Asp + Pro127Asp + Ala131Gln + Ala161Asp  
 + Asn198Ser  
 Asp 58Glu + Ser126Asp + Gly157Asn + Thr207Ser + Leu211Asp  
 + Gly213Asp  
 Ser104Asp + Leu124Gly + Asn153Asp + Ser182Glu + Asn198Glu  
 + Thr207Asn  
 15 Gly 95Ser + Ser103Glu + Pro129Glu + Ser160Asp + Gln200Asp  
 + Leu211Pro  
 Gly 59Glu + Gly125Asp + Pro127Glu + Ala131His + Ala209Asp  
 + Leu211Ile  
 Pro127Glu + Gly157Asn + Pro204Gly + Thr207Asp + Ala209Asp  
 20 + Thr214Asp  
 Gln 57Glu + Leu 94Glu + Ser103Asp + Gly152Asp + Gly205Ser  
 + Leu211Ala  
 Ala 96Glu + Ser103Asp + Gly152Gln + Ser158Glu + Ser160Asp  
 + Thr214Asn  
 25 Gly 61Glu + Tyr102His + Asn153Asp + Phe183His + Gln185Asn  
 + Ser210Asp  
 Ser 97Asp + Ser126Asp + Pro129Glu + Val197His + Asn198Asp  
 + Gln200Asn  
 Asn 60Glu + Ala 96Pro + Ile105Asp + Leu124Ala + Ser130Glu  
 30 + Ile159Ser  
 Gln 57Glu + Pro127Gly + Ser128Glu + Ala131Ser + Ser210Asp  
 + Asn212Glu  
 Ala 96Glu + Tyr102Asp + Ser128Glu + Ala156Pro + Tyr161Val  
 + Leu211Thr  
 35 Gln 57Ser + Asp 58Glu + Ser 99Asp + Gln200Glu + Tyr208Ser  
 + Asn212Glu  
 Gly 59Asp + Leu 94Met + Ile105Ala + Gly152Glu + Ser182Glu  
 + Gln185Ser  
 Asn 60Glu + Ala156Gly + Val197Asp + Asn198Ser + Gln200Glu  
 40 + Ser206Asp  
 Gly100Ser + Ser101Asp + Gly125Gln + Ser126Asp + Pro127Asn  
 + Asn198Glu  
 Ala 96Glu + Ser 99Asp + Ser160Asp + Ala161Gly + Gly205Ser  
 + Ser210Asp  
 45 Leu124Asp + Gly125Pro + Ser130Glu + Ser154Asp + Gly205Gln  
 + Ala209Thr  
 Ser101Glu + Gly152Glu + Gly155Glu + Asn198Gln + Gln200Asp  
 + Asn212Ser  
 Gly 95Asn + Ser103Asp + Gly152Glu + Gly155Asp + Tyr208Val  
 50 + Ser210Glu  
 Ser 99Glu + Gly100Ser + Gly125Asn + Gly152Asp + Gly155Asp  
 + Gly205Asp  
 Ala 96Glu + Ser160Asp + Tyr161Met + Phe183Leu + Gln185Asp  
 + Gly205Asp

Leu 94Glu + Ser104Asp + Gly157Glu + Asn198Gln + Thr207Asn  
 + Ala209Asp  
 Thr 64Gln + Ser101Glu + Ile105Pro + Gly125Ser + Pro129Glu  
 + Thr214Asn  
 5 Gly 98Gln + Tyr102Gln + Gly125Asn + Ser126Glu + Ser158Glu  
 + Asn198Glu  
 Leu 94Asp + Tyr161Ile + Ala181Gly + Phe183Asp + Gln200Glu  
 + Thr214Asp

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TABLE 38  
 Preferred Subtilisin 309 Variants

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--Single Mutation--  
 Thr207Glu  
 15 Ser210Glu  
 Ser210Asp  
 Ser210Gly  
 Val197Glu

--Double Mutation--  
 20 Gln200Glu + Ser210Glu  
 Val199Leu + Ser210Glu  
 Val199Leu + Ser210Asp  
 Pro204Ala + Ala209Thr  
 25 Thr207Glu + Ser210Glu  
 Tyr208Phe + Leu211Asn  
 Ala194Glu + Ser210Glu  
 Gln200Glu + Tyr211Asn  
 Gln206Glu + Thr207Glu

30 --Triple Mutation--  
 Gln200Pro + Gly205Ala + Ser210Glu  
 Thr207Glu + Ser210Glu + leu211Asn  
 Val199Ile + Pro204Asn + Thr207Glu  
 35 Gln200Glu + Ser210Glu + Leu211Asp  
 Gln200Glu + Thr207Glu + Leu211Glu  
 Gln200Glu + Thr207Gln + Ser210Glu

--Quadruple Mutation--  
 40 Pro204Ala + Thr207Glu + Ser210Glu + Leu211Asn  
 Gln206Glu + Thr207Glu + Ser210Glu + Leu211Gly

--Quintuple Mutation--  
 45 Val197Leu + Pro204Ala + Thr207Glu + Ser210Glu + Leu217Asp  
 Asn198Glu + Gln200Glu + Thr207Glu + Ser210Glu + Leu211Asp

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## II. Cleaning Compositions

In another embodiment of the present invention, an effective amount of one or more enzyme variants of the present invention are included in  
 50 compositions useful for cleaning a variety of surfaces in need of proteinaceous



stain removal. Such cleaning compositions include detergent compositions for cleaning hard surfaces, unlimited in form (e.g., liquid and granular); detergent compositions for cleaning fabrics, unlimited in form (e.g., granular, liquid and bar formulations); dishwashing compositions (unlimited in form); oral cleaning compositions, unlimited in form (e.g., dentifrice, toothpaste and mouthwash formulations); denture cleaning compositions, unlimited in form (e.g., liquid, tablet); and contact lens cleaning compositions, unlimited in form (e.g., liquid, tablet). As used herein, "effective amount of enzyme variant" refers to the quantity of enzyme variant necessary to achieve the enzymatic activity necessary in the specific cleaning composition. Such effective amounts are readily ascertained by one of ordinary skill in the art and is based on many factors, such as the particular enzyme variant used, the cleaning application, the specific composition of the cleaning composition, and whether a liquid or dry (e.g., granular, bar) composition is required, and the like. Preferably the cleaning compositions of the present invention comprise from about 0.0001% to about 10% of one or more enzyme variants of the present invention, more preferably from about 0.001% to about 1%, more preferably still from about 0.01% to about 0.1%. Several examples of various cleaning compositions wherein the enzyme variants of the present invention may be employed are discussed in further detail below. All parts, percentages and ratios used herein are by weight unless otherwise specified.

As used herein, "non-fabric cleaning compositions" include hard surface cleaning compositions, dishwashing compositions, oral cleaning compositions, denture cleaning compositions and contact lens cleaning compositions.

25 A. Cleaning Compositions for Hard Surfaces, Dishes and Fabrics

The enzymes of the present invention can be used in any detergent composition where high sudsing and good insoluble substrate removal are desired. Thus the enzyme variants of the present invention can be used with various conventional ingredients to provide fully-formulated hard-surface cleaners, dishwashing compositions, fabric laundering compositions and the like. Such compositions can be in the form of liquids, granules, bars and the like. Such compositions can be formulated as modern "concentrated" detergents which contain as much as 30%-60% by weight of surfactants.

The cleaning compositions herein can optionally, and preferably, contain various anionic, nonionic, zwitterionic, etc., surfactants. Such surfactants are typically present at levels of from about 5% to about 35% of the compositions.

Nonlimiting examples of surfactants useful herein include the

conventional C<sub>11</sub>-C<sub>18</sub> alkyl benzene sulfonates and primary and random alkyl sulfates, the C<sub>10</sub>-C<sub>18</sub> secondary (2,3) alkyl sulfates of the formulas CH<sub>3</sub>(CH<sub>2</sub>)<sub>x</sub>(CHOSO<sub>3</sub><sup>-</sup>M<sup>+</sup>)CH<sub>3</sub> and CH<sub>3</sub>(CH<sub>2</sub>)<sub>y</sub>(CHOSO<sub>3</sub><sup>-</sup>M<sup>+</sup>)CH<sub>2</sub>CH<sub>3</sub> wherein x and (y+1) are integers of at least about 7, preferably at least about 9, and M is a water-solubilizing cation, especially sodium, the C<sub>10</sub>-C<sub>18</sub> alkyl alkoxy sulfates (especially EO 1-5 ethoxy sulfates), C<sub>10</sub>-C<sub>18</sub> alkyl alkoxy carboxylates (especially the EO 1-5 ethoxycarboxylates), the C<sub>10</sub>-C<sub>18</sub> alkyl polyglycosides, and their corresponding sulfated polyglycosides, C<sub>12</sub>-C<sub>18</sub> alpha-sulfonated fatty acid esters, C<sub>12</sub>-C<sub>18</sub> alkyl and alkyl phenol alkoxyates (especially ethoxylates and mixed ethoxy/propoxy), C<sub>12</sub>-C<sub>18</sub> betaines and sulfobetaines ("sultaines"), C<sub>10</sub>-C<sub>18</sub> amine oxides, and the like. The alkyl alkoxy sulfates (AES) and alkyl alkoxy carboxylates (AEC) are preferred herein. (Use of such surfactants in combination with the aforesaid amine oxide and/or betaine or sultaine surfactants is also preferred, depending on the desires of the formulator.) Other conventional useful surfactants are listed in standard texts. Particularly useful surfactants include the C<sub>10</sub>-C<sub>18</sub> N-methyl glucamides disclosed in US Patent 5, 194,639, Connor et al., issued March 16, 1993, incorporated herein by reference.

A wide variety of other ingredients useful in detergent cleaning compositions can be included in the compositions herein, including other active ingredients, carriers, hydrotropes, processing aids, dyes or pigments, solvents for liquid formulations, etc. If an additional increment of sudsing is desired, suds boosters such as the C<sub>10</sub>-C<sub>16</sub> alkylamides can be incorporated into the compositions, typically at about 1% to about 10% levels. The C<sub>10</sub>-C<sub>14</sub> monoethanol and diethanol amides illustrate a typical class of such suds boosters. Use of such suds boosters with high sudsing adjunct surfactants such as the amine oxides, betaines and sultaines noted above is also advantageous. If desired, soluble magnesium salts such as MgCl<sub>2</sub>, MgSO<sub>4</sub>, and the like, can be added at levels of, typically, from about 0.1% to about 2%, to provide additionally sudsing.

The liquid detergent compositions herein can contain water and other solvents as carriers. Low molecular weight primary or secondary alcohols exemplified by methanol, ethanol, propanol, and isopropanol are suitable. Monohydric alcohols are preferred for solubilizing surfactants, but polyols such as those containing from about 2 to about 6 carbon atoms and from about 2 to about 6 hydroxy groups (e.g., 1,3-propanediol, ethylene glycol, glycerine, and 1,2-propanediol) can also be used. The compositions may contain from about 5% to about 90%, typically from about 10% to about 50% of such carriers.

The detergent compositions herein will preferably be formulated such that during use in aqueous cleaning operations, the wash water will have a pH between about 6.8 and about 11.0. Finished products thus are typically formulated at this range. Techniques for controlling pH at recommended  
5 usage levels include the use of buffers, alkalis, acids, etc., and are well known to those skilled in the art.

When formulating the hard surface cleaning compositions and fabric cleaning compositions of the present invention, the formulator may wish to employ various builders at levels from about 5% to about 50% by weight.  
10 Typical builders include the 1-10 micron zeolites, polycarboxylates such as citrate and oxydisuccinates, layered silicates, phosphates, and the like. Other conventional builders are listed in standard formularies.

Likewise, the formulator may wish to employ various additional enzymes, such as cellulases, lipases, amylases and proteases in such  
15 compositions, typically at levels of from about 0.001% to about 1% by weight. Various detergent and fabric care enzymes are well-known in the laundry detergent art.

Various bleaching compounds, such as the percarbonates, perborates and the like, can be used in such compositions, typically at levels from about  
20 1% to about 15% by weight. If desired, such compositions can also contain bleach activators such as tetraacetyl ethylenediamine, nonanoyloxybenzene sulfonate, and the like, which are also known in the art. Usage levels typically range from about 1% to about 10% by weight.

Various soil release agents, especially of the anionic oligoester type,  
25 various chelating agents, especially the aminophosphonates and ethylenediaminedisuccinates, various clay soil removal agents, especially ethoxylated tetraethylene pentamine, various dispersing agents, especially polyacrylates and polyaspartates, various brighteners, especially anionic brighteners, various suds suppressors, especially silicones and secondary  
30 alcohols, various fabric softeners, especially smectite clays, and the like can all be used in such compositions at levels ranging from about 1% to about 35% by weight. Standard formularies and published patents contain multiple, detailed descriptions of such conventional materials.

Enzyme stabilizers may also be used in the cleaning compositions of  
35 the present invention. Such enzyme stabilizers include propylene glycol (preferably from about 1% to about 10%), sodium formate (preferably from about 0.1% to about 1%) and calcium formate (preferably from about 0.1% to about 1%).

1. Hard surface cleaning compositions

As used herein "hard surface cleaning composition" refers to liquid and granular detergent compositions for cleaning hard surfaces such as floors, walls, bathroom tile, and the like. Hard surface cleaning compositions of the present invention comprise an effective amount of one or more enzyme variants of the present invention, preferably from about 0.001% to about 10%, more preferably from about .01% to about 5%, more preferably still from about .05% to about 1% by weight of active enzyme of the composition. In addition to comprising one or more enzyme variants of the present invention, such hard surface cleaning compositions typically comprise a surfactant and a water-soluble sequestering builder. In certain specialized products such as spray window cleaners, however, the surfactants are sometimes not used since they may produce a filmy/streaky residue on the glass surface.

The surfactant component, when present, may comprise as little as 0.1% of the compositions herein, but typically the compositions will contain from about 0.25% to about 10%, more preferably from about 1% to about 5% of surfactant.

Typically the compositions will contain from about 0.5% to about 50% of a detergency builder, preferably from about 1% to about 10%. Preferably the pH should be in the range of about 8 to 12. Conventional pH adjustment agents such as sodium hydroxide, sodium carbonate or hydrochloric acid can be used if adjustment is necessary.

Solvents may be included in the compositions. Useful solvents include, but are not limited to, glycol ethers such as diethyleneglycol monohexyl ether, diethyleneglycol monobutyl ether, ethyleneglycol monobutyl ether, ethyleneglycol monohexyl ether, propyleneglycol monobutyl ether, dipropyleneglycol monobutyl ether, and diols such as 2,2,4-trimethyl-1,3-pentanediol and 2-ethyl-1,3-hexanediol. When used, such solvents are typically present at levels of from about 0.5% to about 15%, preferably from about 3% to about 11%.

Additionally, highly volatile solvents such as isopropanol or ethanol can be used in the present compositions to facilitate faster evaporation of the composition from surfaces when the surface is not rinsed after "full strength" application of the composition to the surface. When used, volatile solvents are typically present at levels of from about 2% to about 12% in the compositions.

The hard surface cleaning composition embodiment of the present invention is illustrated by the following examples.

## Examples 7-12

Liquid Hard Surface Cleaning Compositions							
		Example No.					
	Component	7	8	9	10	11	12
5	Thr207Glu	0.05	0.50	0.02	0.03	0.10	0.03
	Val199Leu + Ser210Asp	—	—	—	—	0.20	0.02
	Na <sub>2</sub> DIDA*						
	EDTA**	—	—	2.90	2.90	—	—
	Na Citrate	—	—	—	—	2.90	2.90
10	NaC <sub>12</sub> Alkyl-benzene sulfonate	1.95	—	1.95	—	1.95	—
	NaC <sub>12</sub> Alkylsulfate	—	2.20	—	2.20	—	2.20
	NaC <sub>12</sub> (ethoxy)*** sulfate	—	2.20	—	2.20	—	2.20
15	C <sub>12</sub> Dimethylamine oxide	—	0.50	—	0.50	—	0.50
	Na Cumene sulfonate	1.30	—	1.30	—	1.30	—
	Hexyl Carbitol***	6.30	6.30	6.30	6.30	6.30	6.30
	Water****	balance to 100%					
20	*Disodium N-diethyleneglycol-N,N-iminodiacetate						
	**Na <sub>4</sub> ethylenediamine diacetic acid						
	***Diethyleneglycol monohexyl ether						
	****All formulas adjusted to pH 7						

In Examples 7-10, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Thr207Glu, with substantially similar results.

In Examples 11-12, any combination of the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Val199Leu + Ser210Asp, with substantially similar results.

Examples 13-18  
Spray Compositions for Cleaning Hard Surfaces  
and Removing Household Mildew

5	Component	Example No.					
		13	14	15	16	17	18
	Gln200Glu + Thr207Glu	0.50	0.05	0.60	0.30	0.20	0.30
	Ser210Glu	-	-	-	-	0.30	0.10
	Sodium octyl sulfate	2.00	2.00	2.00	2.00	2.00	2.00
	Sodium dodecyl sulfate	4.00	4.00	4.00	4.00	4.00	4.00
10	Sodium hydroxide	0.80	0.80	0.80	0.80	0.80	0.80
	Silicate (Na)	0.04	0.04	0.04	0.04	0.04	0.04
	Perfume	0.35	0.35	0.35	0.35	0.35	0.35
	Water	balance to 100%					

Product pH is about 7.

15 In Examples 13-16, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Gln200Glu + Thr207Glu, with substantially similar results.

In Examples 17-18, any combination of the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Gln200Glu +  
20 Thr207Glu and Ser210Glu, with substantially similar results.

## 2. Dishwashing Compositions

In another embodiment of the present invention, dishwashing compositions comprise one or more enzyme variants of the present invention. As used herein, "dishwashing composition" refers to all forms for compositions  
25 for cleaning dishes, including but not limited to, granular and liquid forms. The dishwashing composition embodiment of the present invention is illustrated by the following examples.

Examples 19-24  
Dishwashing Composition

		Example No.					
Component		19	20	21	22	23	24
5	Gln200Pro + Gly205Ala + Ser210Asp	0.05	0.50	0.02	0.40	0.10	0.03
	Val199Leu + Ser210Asp	-	-	-	-	0.40	0.02
	C <sub>12</sub> -C <sub>14</sub> N-methyl- glucamide	0.90	0.90	0.90	0.90	0.90	0.90
10	C <sub>12</sub> ethoxy (1) sulfate	12.00	12.00	12.00	12.00	12.00	12.00
	2-methyl undecanoic acid	4.50	4.50	4.50	4.50	4.50	4.50
	C <sub>12</sub> ethoxy (2) carboxylate	4.50	4.50	4.50	4.50	4.50	4.50
	C <sub>12</sub> alcohol ethoxylate (4)	3.00	3.00	3.00	3.00	3.00	3.00
	C <sub>12</sub> amine oxide	3.00	3.00	3.00	3.00	3.00	3.00
15	Sodium cumene sulfonate	2.00	2.00	2.00	2.00	2.00	2.00
	Ethanol	4.00	4.00	4.00	4.00	4.00	4.00
	Mg <sup>++</sup> (as MgCl <sub>2</sub> )	0.20	0.20	0.20	0.20	0.20	0.20
	Ca <sup>++</sup> (as CaCl <sub>2</sub> )	0.40	0.40	0.40	0.40	0.40	0.40
	Water	balance to 100%					

20 Product pH is adjusted to 7.

In Examples 19-22, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Gln200Pro + Gly205Ala + Ser210Asp, with substantially similar results.

25 In Examples 23-24, any combination of the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Gln200Pro + Gly205Ala + Ser210Asp and Val199Leu + Ser210Asp, with substantially similar results.

### 3. Fabric cleaning compositions

30 In another embodiment of the present invention, fabric cleaning compositions comprise one or more enzyme variants of the present invention. As used herein, "fabric cleaning composition" refers to all forms for detergent compositions for cleaning fabrics, including but not limited to, granular, liquid and bar forms. Preferred fabric cleaning compositions are those in the liquid form.

#### 35 a. Granular fabric cleaning compositions

The granular fabric cleaning compositions of the present invention contain an effective amount of one or more enzyme variants of the present invention, preferably from about 0.001% to about 10%, more preferably from

about 0.005% to about 5%, more preferably from about 0.01% to about 1% by weight of active enzyme of the composition. In addition to one or more enzyme variants, the granular fabric cleaning compositions typically comprise at least one surfactant, one or more builders, and, in some cases, a bleaching agent.

The granular fabric cleaning composition embodiment of the present invention is illustrated by the following examples.

## Examples 25-28

## Granular Fabric Cleaning Composition

Component	Example No.			
	25	26	27	28
Ser99Asp	0.10	0.20	0.03	0.05
Ser99Gly	-	-	0.02	0.05
C <sub>13</sub> linear alkyl benzene sulfonate	22.00	22.00	22.00	22.00
Phosphate (as sodium tripolyphosphates)	23.00	23.00	23.00	23.00
Sodium carbonate	23.00	23.00	23.00	23.00
Sodium silicate	14.00	14.00	14.00	14.00
Zeolite	8.20	8.20	8.20	8.20
Chelant (diethylenetriamine-pentaacetic acid)	0.40	0.40	0.40	0.40
Sodium sulfate	5.50	5.50	5.50	5.50
Water	balance to 100%			

In Examples 25-26, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Ser99Asp, with substantially similar results.

In Examples 27-28, any combination of the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Ser99Asp and Ser99Gly, with substantially similar results.



Examples 29-32  
Granular Fabric Cleaning Composition

	Component	Example No.			
		29	30	31	32
5	Gln200Glu + Thr207Glu + Ser210Glu	0.10	0.20	0.03	0.05
	Asn74Asp + Pro204Ala + Thr207Glu	-	-	0.02	0.05
	C <sub>12</sub> alkyl benzene sulfonate	12.00	12.00	12.00	12.00
	Zeolite A (1-10 micrometer)	26.00	26.00	26.00	26.00
	2-butyl octanoic acid	4.00	4.00	4.00	4.00
10	C <sub>12</sub> -C <sub>14</sub> secondary (2,3) alkyl sulfate, Na salt	5.00	5.00	5.00	5.00
	Sodium citrate	5.00	5.00	5.00	5.00
	Optical brightener	0.10	0.10	0.10	0.10
	Sodium sulfate	17.00	17.00	17.00	17.00
15	Water and minors	balance to 100%			

In Examples 29-30, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Gln200Glu + Thr207Glu + Ser210Glu, with substantially similar results.

In Examples 31-32, any combination of the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Gln200Glu + Thr207Glu + Ser210Glu and Asn74Asp + Pro204Ala + Thr207Glu, with substantially similar results.

Examples 33-36  
Granular Fabric Cleaning Composition

	Component	Example No.			
		33	34	35	36
25	Leu 94Gly + Gln200Glu	0.10	0.20	0.03	0.05
	Gln 57Ser + Leu 94Gly + Gln200Glu	-	-	0.02	0.05
	C <sub>13</sub> linear alkyl benzene sulfonate	22.00	22.00	22.00	22.00
30	Phosphate (as sodium tripolyphosphates)	23.00	23.00	23.00	23.00
	Sodium carbonate	23.00	23.00	23.00	23.00
	Sodium silicate	14.00	14.00	14.00	14.00
	Zeolite	8.20	8.20	8.20	8.20
35	Chelant (diethylenetriamine-pentaacetic acid)	0.40	0.40	0.40	0.40
	Sodium sulfate	5.50	5.50	5.50	5.50
	Water	balance to 100%			

In Examples 33-34, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Leu 94Gly + Gln200Glu, with substantially similar results.

In Examples 35-36, any combination of the subtilisin 309 variants  
5 recited in Tables 3-38, among others, are substituted for Leu 94Gly + Gln200Glu and Gln 57Ser + Leu 94Gly + Gln200Glu, with substantially similar results.

## Examples 37-40

## Granular Fabric Cleaning Composition

Component	Example No.			
	37	38	39	40
Asn74His + Gln 57Ser + Asn 60Ser + Leu 94Gly + Gln200Glu	0.10	0.20	0.03	0.05
Val 93Gln + Tyr102Cys + Ser154Glu + Asn198Gln + Thr207Gly	-	-	0.02	0.05
C <sub>12</sub> alkyl benzene sulfonate	12.00	12.00	12.00	12.00
Zeolite A (1-10 micrometer)	26.00	26.00	26.00	26.00
2-butyl octanoic acid	4.00	4.00	4.00	4.00
C <sub>12</sub> -C <sub>14</sub> secondary (2,3) alkyl sulfate, Na salt	5.00	5.00	5.00	5.00
Sodium citrate	5.00	5.00	5.00	5.00
Optical brightener	0.10	0.10	0.10	0.10
Sodium sulfate	17.00	17.00	17.00	17.00
Water and minors	balance to 100%			

25 In Examples 37-38, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Asn74His + Gln 57Ser + Asn 60Ser + Leu 94Gly + Gln200Glu, with substantially similar results.

In Examples 39-40, any combination of the subtilisin 309 variants  
30 recited in Tables 3-38, among others, are substituted for Asn74His + Gln 57Ser + Asn 60Ser + Leu 94Gly + Gln200Glu and Val 93Gln + Tyr102Cys + Ser154Glu + Asn198Gln + Thr207Gly, with substantially similar results.

Examples 41-42  
Granular Fabric Cleaning Composition

		Example No.	
Component		41	42
5	Linear alkyl benzene sulphonate	11.4	10.70
	Tallow alkyl sulphate	1.80	2.40
	C <sub>14-15</sub> alkyl sulphate	3.00	3.10
	C <sub>14-15</sub> alcohol 7 times ethoxylated	4.00	4.00
	Tallow alcohol 11 times ethoxylated	1.80	1.80
10	Dispersant	0.07	0.1
	Silicone fluid	0.80	0.80
	Trisodium citrate	14.00	15.00
	Citric acid	3.00	2.50
	Zeolite	32.50	32.10
15	Maleic acid acrylic acid copolymer	5.00	5.00
	Diethylene triamine penta methylene phosphonic acid	1.00	0.20
	Ser210Asp	0.30	0.30
	Lipase	0.36	0.40
20	Amylase	0.30	0.30
	Sodium silicate	2.00	2.50
	Sodium sulphate	3.50	5.20
	Polyvinyl pyrrolidone	0.30	0.50
	Perborate	0.5	1
25	Phenol sulphonate	0.1	0.2
	Peroxidase	0.1	0.1
	Minors	Up to 100	Up to 100

Examples 43-44  
Granular Fabric Cleaning Composition

		Example No.	
Component		43	44
5	Sodium linear C <sub>12</sub> alkyl benzene-sulfonate	6.5	8.0
	Sodium sulfate	15.0	18.0
	Zeolite A	26.0	22.0
	Sodium nitrilotriacetate	5.0	5.0
	Polyvinyl pyrrolidone	0.5	0.7
10	Tetraacetylene diamine	3.0	3.0
	Boric acid	4.0	-
	Perborate	0.5	1
	Phenol sulphonate	0.1	0.2
	Val199Leu + Ser210Glu	0.4	0.4
15	Fillers (e.g., silicates; carbonates; perfumes; water)	Up to 100	Up to 100

Example 45  
Compact Granular Fabric Cleaning Composition

Component	Weight %
Alkyl Sulphate	8.0
Alkyl Ethoxy Sulphate	2.0
Mixture of C <sub>25</sub> and C <sub>45</sub> alcohol 3 and 7 times ethoxylated	6.0
Polyhydroxy fatty acid amide	2.5
Zeolite	17.0
Layered silicate/citrate	16.0
Carbonate	7.0
Maleic acid acrylic acid copolymer	5.0
Soil release polymer	0.4
Carboxymethyl cellulose	0.4
Poly (4-vinylpyridine) -N-oxide	0.1
Copolymer of vinylimidazole and vinylpyrrolidone	0.1
PEG2000	0.2
Asn74Asp + Val197Glu + Gln200Glu + Ser210Glu	0.5
Lipase	0.2
Cellulase	0.2
Tetracetylene diamine	6.0
Percarbonate	22.0
Ethylene diamine disuccinic acid	0.3

Suds suppressor	3.5
Disodium-4,4'-bis (2-morpholino -4-anilino-s-triazin-6-ylamino) stilbene-2,2'-disulphonate	0.25
Disodium-4,4'-bis (2-sulfostyryl) biphenyl	0.05
Water, Perfume and Minors	Up to 100

## Example 46

## Granular Fabric Cleaning Composition

Component	Weight %
Linear alkyl benzene sulphonate	7.6
C <sub>16</sub> -C <sub>18</sub> alkyl sulfate	1.3
C <sub>14-15</sub> alcohol 7 times ethoxylated	4.0
Coco-alkyl-dimethyl hydroxyethyl ammonium chloride	1.4
Dispersant	0.07
Silicone fluid	0.8
Trisodium citrate	5.0
Zeolite 4A	15.0
Maleic acid acrylic acid copolymer	4.0
Diethylene triamine penta methylene phosphonic acid	0.4
Perborate	15.0
Tetraacetylene diamine	5.0
Smectite clay	10.0
Poly (oxy ethylene) (MW 300,000)	0.3
Tyr208Phe + Leu211Asn	0.4
Lipase	0.2
Amylase	0.3
Cellulase	0.2
Sodium silicate	3.0
Sodium carbonate	10.0
Carboxymethyl cellulose	0.2
Brighteners	0.2
Water, perfume and minors	Up to 100

## Example 47

## Granular Fabric Cleaning Composition

Component	Weight %
Linear alkyl benzene sulfonate	6.92
Tallow alkyl sulfate	2.05
C <sub>14-15</sub> alcohol 7 times ethoxylated	4.4
C <sub>12-15</sub> alkyl ethoxy sulfate - 3 times ethoxylated	0.16
Zeolite	20.2
Citrate	5.5
Carbonate	15.4
Silicate	3.0
Maleic acid acrylic acid copolymer	4.0
Carboxymethyl cellulase	0.31
Soil release polymer	0.30
Asn74His + Val197Glu + Pro204Ala + Ala209Thr + Ser210Glu	0.2
Lipase	0.36
Cellulase	0.13
Perborate tetrahydrate	11.64
Perborate monohydrate	8.7
Tetraacetylene diamine	5.0
Diethylene tramine penta methyl phosphonic acid	0.38
Magnesium sulfate	0.40
Brightener	0.19
Perfume, silicone, suds suppressors	0.85
Minors	Up to 100

b. Liquid fabric cleaning compositions

- 5 Liquid fabric cleaning compositions of the present invention comprise an effective amount of one or more enzyme variants of the present invention, preferably from about 0.005% to about 5%, more preferably from about 0.01% to about 1%, by weight of active enzyme of the composition. Such liquid fabric cleaning compositions typically additionally comprise an anionic surfactant, a
- 10 fatty acid, a water-soluble detergency builder and water.

The liquid fabric cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 48-52  
Liquid Fabric Cleaning Compositions

		Example No.				
Component		48	49	50	51	52
5	Pro204Ala + Ala209Thr	0.05	0.03	0.30	0.03	0.10
	Gln200Glu + Thr207Glu + Ser210Glu	-	-	-	0.01	0.20
	C <sub>12</sub> -C <sub>14</sub> alkyl sulfate, Na	20.00	20.00	20.00	20.00	20.00
	2-butyl octanoic acid	5.00	5.00	5.00	5.00	5.00
10	Sodium citrate	1.00	1.00	1.00	1.00	1.00
	C <sub>10</sub> alcohol ethoxylate (3)	13.00	13.00	13.00	13.00	13.00
	Monethanolamine	2.50	2.50	2.50	2.50	2.50
	Water/propylene glycol/ethanol (100:1:1)	balance to 100%				

15 In Examples 48-50 the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Pro204Ala + Ala209Thr, with substantially similar results.

20 In Examples 51-52, any combination of the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Pro204Ala + Ala209Thr and Gln200Glu + Thr207Glu + Ser210Glu, with substantially similar results.

Examples 53-57  
Liquid Fabric Cleaning Compositions

		Example No.				
Component		53	54	55	56	57
25	Tyr102Cys + Ile105Val + Leu124Ile + Ser154Glu + Asn198Gln + Thr207Gly	0.05	0.03	0.30	0.03	0.10
	Asn74Asp + Ser97Asp + Gln 57Ser + Asn 60Ser	-	-	-	0.01	0.20
30	C <sub>12</sub> -C <sub>14</sub> alkyl sulfate, Na	20.00	20.00	20.00	20.00	20.00
	2-butyl octanoic acid	5.00	5.00	5.00	5.00	5.00
	Sodium citrate	1.00	1.00	1.00	1.00	1.00
	C <sub>10</sub> alcohol ethoxylate (3)	13.00	13.00	13.00	13.00	13.00
	Monethanolamine	2.50	2.50	2.50	2.50	2.50
35	Water/propylene glycol/ethanol (100:1:1)	balance to 100%				

In Examples 53-55 the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Tyr102Cys + Ile105Val + Leu124Ile + Ser154Glu + Asn198Gln + Thr207Gly, with substantially similar results.

In Examples 56-57, any combination of the subtilisin 309 variants recited in Tables 212, among others, are substituted for Tyr102Cys + Ile105Val + Leu124Ile + Ser154Glu + Asn198Gln + Thr207Gly and Asn74Asp + Ser97Asp + Gln 57Ser + Asn 60Ser, with substantially similar results.

5

## Examples 58-59

## Granular Fabric Cleaning Composition

Component	Example No.	
	58	59
C <sub>12-14</sub> alkenyl succinic acid	3.0	8.0
10 Citric acid monohydrate	10.0	15.0
Sodium C <sub>12-15</sub> alkyl sulphate	8.0	8.0
Sodium sulfate of C <sub>12-15</sub> alcohol 2 times ethoxylated	-	3.0
C <sub>12-15</sub> alcohol 7 times ethoxylated	-	8.0
C <sub>12-15</sub> alcohol 5 times ethoxylated	8.0	-
15 Diethylene triamine penta (methylene phosphonic acid)0.2	-	-
Oleic acid	1.8	-
Ethanol	4.0	4.0
Propanediol	2.0	2.0
Asn74Asp + Ser210Glu	0.2	0.2
20 Polyvinyl pyrrolidone	1.0	2.0
Suds suppressor	0.15	0.15
NaOH	up to pH 7.5	
Perborate	0.5	1
Phenol sulphonate	0.1	0.2
25 Peroxidase	0.4	0.1
Waters and minors	up to 100 parts	

In each of Examples 58 and 59 herein, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Asn74Asp + Ser210Glu, with substantially similar results.



Examples 60-62  
Liquid Fabric Cleaning Composition

		Example No.		
Component		60	61	62
5	Citric Acid	7.10	3.00	3.00
	Fatty Acid	2.00	-	2.00
	Ethanol	1.93	3.20	3.20
	Boric Acid	2.22	3.50	3.50
	Monoethanolamine	0.71	1.09	1.09
10	1,2 Propanediol	7.89	8.00	8.00
	NaCumene Sulfonate	1.80	3.00	3.00
	NaFormate	0.08	0.08	0.08
	NaOH	6.70	3.80	3.80
	Silicon anti-foam agent	1.16	1.18	1.18
15	Ser210Glu	0.0145	-	-
	Asn74Asp + Ser97Asp + Ser210Glu	-	0.0145	-
	Gln200Glu + Ser210Glu	-	-	0.0145
	Lipase	0.200	0.200	0.200
	Cellulase	-	7.50	7.50
20	Soil release polymer	0.29	0.15	0.15
	Anti-foaming agents	0.06	0.085	0.085
	Brightener 36	0.095	-	-
	Brightener 3	-	0.05	0.05
	C <sub>12</sub> alkyl benzenesulfonic acid	9.86	-	-
25	C <sub>12-15</sub> alkyl polyethoxylate (2.5) sulfate	13.80	18.00	18.00
	C <sub>12</sub> glucose amide	-	5.00	5.00
	C <sub>12-13</sub> alkyl polyethoxylate (9)	2.00	2.00	2.00
	Water, perfume and minors	balance to 100%		

c. Bar fabric cleaning compositions

30 Bar fabric cleaning compositions of the present invention suitable for hand-washing soiled fabrics contain an effective amount of one or more enzyme variants of the present invention, preferably from about 0.001% to about 10%, more preferably from about 0.01% to about 1% by weight of the composition.

35 The bar fabric cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 63-66  
Bar Fabric Cleaning Compositions

		Example No.			
Component		63	64	65	66
5	Val197Glu	0.3	-	0.1	0.02
	Tyr208Phe + Leu211Asn	-	-	0.4	0.03
	C <sub>12</sub> -C <sub>16</sub> alkyl sulfate, Na	20.0	20.0	20.0	20.00
	C <sub>12</sub> -C <sub>14</sub> N-methyl glucamide	5.0	5.0	5.0	5.00
	C <sub>11</sub> -C <sub>13</sub> alkyl benzene sulfonate, Na	10.0	10.0	10.0	10.00
10	Sodium carbonate	25.0	25.0	25.0	25.00
	Sodium pyrophosphate	7.0	7.0	7.0	7.00
	Sodium tripolyphosphate	7.0	7.0	7.0	7.00
	Zeolite A (0.1-10 $\mu$ )	5.0	5.0	5.0	5.00
	Carboxymethylcellulose	0.2	0.2	0.2	0.20
15	Polyacrylate (MW 1400)	0.2	0.2	0.2	0.20
	Coconut monethanolamide	5.0	5.0	5.0	5.00
	Brightener, perfume	0.2	0.2	0.2	0.20
	CaSO <sub>4</sub>	1.0	1.0	1.0	1.00
	MgSO <sub>4</sub>	1.0	1.0	1.0	1.00
20	Water	4.0	4.0	4.0	4.00
	Filler*	balance to 100%			

\*Can be selected from convenient materials such as CaCO<sub>3</sub>, talc, clay, silicates, and the like.

In Examples 63-64 the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Val197Glu, with substantially similar results.

In Examples 65-66, any combination of the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Val197Glu and Tyr208Phe + Leu211Asn, with substantially similar results.

Examples 67-70  
Bar Fabric Cleaning Compositions

		Example No.			
Component		67	68	69	70
5	Asn 60Ser + Val 93Gln + Gly213Asp	0.3	-	0.1	0.02
	Val 93Gln + Tyr102Cys + Thr207Gly + Gly213Asp	-	0.3	0.4	0.03
	C <sub>12</sub> -C <sub>16</sub> alkyl sulfate, Na	20.0	20.0	20.0	20.00
	C <sub>12</sub> -C <sub>14</sub> N-methyl glucamide	5.0	5.0	5.0	5.00
10	C <sub>11</sub> -C <sub>13</sub> alkyl benzene sulfonate, Na	10.0	10.0	10.0	10.00
	Sodium carbonate	25.0	25.0	25.0	25.00
	Sodium pyrophosphate	7.0	7.0	7.0	7.00
	Sodium tripolyphosphate	7.0	7.0	7.0	7.00
	Zeolite A (0.1-10 $\mu$ )	5.0	5.0	5.0	5.00
15	Carboxymethylcellulose	0.2	0.2	0.2	0.20
	Polyacrylate (MW 1400)	0.2	0.2	0.2	0.20
	Coconut monethanolamide	5.0	5.0	5.0	5.00
	Brightener, perfume	0.2	0.2	0.2	0.20
	CaSO <sub>4</sub>	1.0	1.0	1.0	1.00
20	MgSO <sub>4</sub>	1.0	1.0	1.0	1.00
	Water	4.0	4.0	4.0	4.00
	Filler*	balance to 100%			

\*Can be selected from convenient materials such as CaCO<sub>3</sub>, talc, clay, silicates, and the like.

25 In Example 67, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Asn 60Ser + Val 93Gln + Gly213Asp, with substantially similar results.

In Example 68, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Val 93Gln + Tyr102Cys + Thr207Gly + Gly213Asp, with substantially similar results.

30 In Examples 69-70, any combination of the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Asn 60Ser + Val 93Gln + Gly213Asp and Val 93Gln + Tyr102Cys + Thr207Gly + Gly213Asp, with substantially similar results.

35 B. Additional Cleaning Compositions

In addition to the hard surface cleaning, dishwashing and fabric cleaning compositions discussed above, one or more enzyme variants of the present invention may be incorporated into a variety of other cleaning

compositions where hydrolysis of an insoluble substrate is desired. Such additional cleaning compositions include but are not limited to, oral cleaning compositions, denture cleaning compositions, and contact lens cleaning compositions.

5 1. Oral cleaning compositions

In another embodiment of the present invention, a pharmaceutically-acceptable amount of one or more enzyme variants of the present invention are included in compositions useful for removing proteinaceous stains from teeth or dentures. As used herein, "oral cleaning compositions" refers to  
10 dentifrices, toothpastes, toothgels, toothpowders, mouthwashes, mouth sprays, mouth gels, chewing gums, lozenges, sachets, tablets, biogels, prophylaxis pastes, dental treatment solutions, and the like. Preferably, the oral cleaning compositions comprise from about 0.0001% to about 20% of one or more enzyme variants of the present invention, more preferably from about 0.001%  
15 to about 10%, more preferably still from about 0.01% to about 5%, by weight of the composition, and a pharmaceutically-acceptable carrier. As used herein, "pharmaceutically-acceptable" means that drugs, medicaments or inert ingredients which the term describes are suitable for use in contact with the tissues of humans and lower animals without undue toxicity, incompatibility,  
20 instability, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio.

Typically, the pharmaceutically-acceptable oral cleaning carrier components of the oral cleaning compositions of the oral cleaning compositions will generally comprise from about 50% to about 99.99%, preferably from  
25 about 65% to about 99.99%, more preferably from about 65% to about 99%, by weight of the composition.

The pharmaceutically-acceptable carrier components and optional components which may be included in the oral cleaning compositions of the present invention are well known to those skilled in the art. A wide variety of  
30 composition types, carrier components and optional components useful in the oral cleaning compositions are disclosed in U.S. Patent 5,096,700, Seibel, issued March 17, 1992; U.S. Patent 5,028,414, Sampathkumar, issued July 2, 1991; and U.S. Patent 5,028,415, Benedict, Bush and Sunberg, issued July 2, 1991; all of which are incorporated herein by reference.

35 The oral cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 71-74  
Dentifrice Composition

	Component	Example No.			
		71	72	73	74
5	Val199Leu + Pro204Ala + Thr207Glu +Ser210Glu	2.000	3.500	1.500	2.000
	Sorbitol (70% aqueous solution)	35.000	35.000	35.000	35.000
	PEG-6*	1.000	1.000	1.000	1.000
	Silica dental abrasive**	20.000	20.000	20.000	20.000
10	Sodium fluoride	0.243	0.243	0.243	0.243
	Titanium dioxide	0.500	0.500	0.500	0.500
	Sodium saccharin	0.286	0.286	0.286	0.286
	Sodium alkyl sulfate (27.9% aqueous solution)	4.000	4.000	4.000	4.000
15	Flavor	1.040	1.040	1.040	1.040
	Carboxyvinyl Polymer***	0.300	0.300	0.300	0.300
	Carrageenan****	0.800	0.800	0.800	0.800
	Water	balance to 100%			

\*PEG-6 = Polyethylene glycol having a molecular weight of 600.

20 \*\*Precipitated silica identified as Zeodent 119 offered by J.M. Huber.

\*\*\*Carbopol offered by B.F. Goodrich Chemical Company.

\*\*\*\*Iota Carrageenan offered by Hercules Chemical Company.

In Examples 71-74 the subtilisin 309 variants recited in Tables 3-38,  
among others, are substituted for Val199Leu + Pro204Ala + Thr207Glu +  
25 Ser210Glu, with substantially similar results.

Examples 75-78  
Mouthwash Composition

	Component	Example No.			
		75	76	77	78
5	Ser210Asp	3.00	7.50	1.00	5.00
	SDA 40 Alcohol	8.00	8.00	8.00	8.00
	Flavor	0.08	0.08	0.08	0.08
	Emulsifier	0.08	0.08	0.08	0.08
	Sodium Fluoride	0.05	0.05	0.05	0.05
10	Glycerin	10.00	10.00	10.00	10.00
	Sweetener	0.02	0.02	0.02	0.02
	Benzoic acid	0.05	0.05	0.05	0.05
	Sodium hydroxide	0.20	0.20	0.20	0.20
	Dye	0.04	0.04	0.04	0.04
15	Water	balance to 100%			

In Examples 75-78, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Ser210Asp, with substantially similar results.

Examples 79-82  
Lozenge Composition

	Component	Example No.			
		79	80	81	82
20	Tyr208Phe + Leu211Asn	0.01	0.03	0.10	0.02
	Sorbitol	17.50	17.50	17.50	17.50
	Mannitol	17.50	17.50	17.50	17.50
25	Starch	13.60	13.60	13.60	13.60
	Sweetener	1.20	1.20	1.20	1.20
	Flavor	11.70	11.70	11.70	11.70
	Color	0.10	0.10	0.10	0.10
	Corn Syrup	balance to 100%			

In Examples 79-82, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Tyr208Phe + Leu211Asn, with substantially similar results.

Examples 83-86  
Chewing Gum Composition

	Component	Example No.			
		83	84	85	86
5	Val199Met + Pro204Ala + Thr207Glu	0.03	0.02	0.10	0.05
	Sorbitol crystals	38.44	38.40	38.40	38.40
	Paloja-T gum base*	20.00	20.00	20.00	20.00
	Sorbitol (70% aqueous solution)	22.00	22.00	22.00	22.00
	Mannitol	10.00	10.00	10.00	10.00
10	Glycerine	7.56	7.56	7.56	7.56
	Flavor	1.00	1.00	1.00	1.00

\*Supplied by L.A. Dreyfus Company.

In Examples 83-86, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Val199Met + Pro204Ala + Thr207Glu, with substantially similar results.

## 2. Denture cleaning compositions

In another embodiment of the present invention, denture cleaning compositions for cleaning dentures outside of the oral cavity comprise one or more enzyme variants of the present invention. Such denture cleaning compositions comprise an effective amount of one or more of the enzyme variants, preferably from about 0.0001% to about 50% of one or more of the enzyme variants, more preferably from about 0.001% to about 35%, more preferably still from about 0.01% to about 20%, by weight of the composition, and a denture cleansing carrier. Various denture cleansing composition formats such as effervescent tablets and the like are well known in the art (see for example U.S. Patent 5,055,305, Young, incorporated herein by reference), and are generally appropriate for incorporation of one or more of the enzyme variants for removing proteinaceous stains from dentures.

The denture cleaning composition embodiment of the present invention is illustrated by the following examples.

## Examples 87-90

## Two-layer Effervescent Denture Cleansing Tablet

	Component	Example No.			
		87	88	89	90
5	<u>Acidic Layer</u>				
	Ser210Glu	1.0	1.5	0.01	0.05
	Tartaric acid	24.0	24.0	24.00	24.00
	Sodium carbonate	4.0	4.0	4.00	4.00
	Sulphamic acid	10.0	10.0	10.00	10.00
10	PEG 20,000	4.0	4.0	4.00	4.00
	Sodium bicarbonate	24.5	24.5	24.50	24.50
	Potassium persulfate	15.0	15.0	15.00	15.00
	Sodium acid pyrophosphate	7.0	7.0	7.00	7.00
	Pyrogenic silica	2.0	2.0	2.00	2.00
15	TAED*	7.0	7.0	7.00	7.00
	Ricinoleylsulfosuccinate	0.5	0.5	0.50	0.50
	Flavor	1.0	1.0	1.00	1.00
	<u>Alkaline Layer</u>				
	Sodium perborate monohydrate	32.0	32.0	32.00	32.00
20	Sodium bicarbonate	19.0	19.0	19.00	19.00
	EDTA	3.0	3.0	3.00	3.00
	Sodium tripolyphosphate	12.0	12.0	12.00	12.00
	PEG 20,000	2.0	2.0	2.00	2.00
	Potassium persulfate	26.0	26.0	26.00	26.00
25	Sodium carbonate	2.0	2.0	2.00	2.00
	Pyrogenic silica	2.0	2.0	2.00	2.00
	Dye/flavor	2.0	2.0	2.00	2.00

\*Tetraacetylene diamine

In Examples 87-90, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Ser210Glu, with substantially similar results.

### 3. Contact Lens Cleaning Compositions

In another embodiment of the present invention, contact lens cleaning compositions comprise one or more enzyme variants of the present invention. Such contact lens cleaning compositions comprise an effective amount of one or more of the enzyme variants, preferably from about 0.01% to about 50% of one or more of the enzyme variants, more preferably from about 0.01% to about 20%, more preferably still from about 1% to about 5%, by weight of the composition, and a contact lens cleaning carrier. Various contact lens



cleaning composition formats such as tablets, liquids and the like are well known in the art (see for example U.S. Patent 4,863,627, Davies, Meaken and Rees, issued September 5, 1989; U.S. Patent Re. 32,672, Huth, Lam and Kirai, reissued May 24, 1988; U.S. Patent 4,609,493, Schäfer, issued  
 5 September 2, 1986; U.S. Patent, 4,690,793, Ogunbiyi and Smith, issued September 1, 1987; U.S. Patent 4,614,549, Ogunbiyi, Riedhammer and Smith, issued September 30, 1986; and U.S. Patent 4,285,738, Ogata, issued August 25, 1981; each of which are incorporated herein by reference), and are generally appropriate for incorporation of one or more enzyme variants of the  
 10 present invention for removing proteinaceous stains from contact lens.

The contact lens cleaning composition embodiment of the present invention is illustrated by the following examples.

#### Examples 91-94

##### Enzymatic Contact Lens Cleaning Solution

15	Component	Example No.			
		91	92	93	94
	Val199Leu + Ser210Asp	0.01	0.5	0.1	2.0
	Glucose	50.00	50.0	50.0	50.0
	Nonionic surfactant (polyoxyethylene- polyoxypropylene copolymer)	2.00	2.0	2.0	2.0
20	Anionic surfactant (polyoxyethylene- alkylphenylether sodium sulfricester)	1.00	1.0	1.0	1.0
	Sodium chloride	1.00	1.0	1.0	1.0
	Borax	0.30	0.3	0.3	0.3
25	Water	balance to 100%			

In Examples 91-94, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Val199Leu + Ser210Asp, with substantially similar results.

While particular embodiments of the subject invention have been  
 30 described, it will be obvious to those skilled in the art that various changes and modifications of the subject invention can be made without departing from the spirit and scope of the invention. It is intended to cover, in the appended claims, all such modifications that are within the scope of the invention.

## SEQUENCE LISTING

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(ii) TITLE OF INVENTION: SUBTILISIN 309 VARIANTS WITH  
DECREASED ADSORPTION AND  
INCREASED HYDROLYSIS

(iii) NUMBER OF SEQUENCES: 2

## (iv) CORRESPONDENCE ADDRESS:

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## (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

## (vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:  
(B) FILING DATE:  
(C) CLASSIFICATION:

## (viii) ATTORNEY/AGENT INFORMATION:

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## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 269 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala  
1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp  
20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser  
35 40 45

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5 Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr  
 50 55 60  
 His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu  
 65 70 75 80  
 10 Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala  
 85 90 95  
 15 Ser Gly Ser Gly Ser Val Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala  
 100 105 110  
 Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser  
 115 120 125  
 20 Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly  
 130 135 140  
 25 Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser  
 145 150 155 160  
 30 Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln  
 165 170 175  
 35 Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile  
 180 185 190  
 40 Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr  
 195 200 205  
 Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala  
 210 215 220  
 45 Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile  
 225 230 235 240  
 50 Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu  
 245 250 255  
 55 Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg  
 260 265

## (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 275 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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(\*1) SEQUENCE DESCRIPTION: SEQ ID NO:2:

5	Ala Gln Ser Val Pro Tyr Gly Val Ser Gln Ile Lys Ala Pro Ala Leu 1 5 10 15
	His Ser Gln Gly Tyr Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp 20 25 30
10	Ser Gly Ile Asp Ser Ser His Pro Asp Leu Lys Val Ala Gly Gly Ala 35 40 45
	Ser Met Val Pro Ser Gln Thr Asn Pro Phe Gln Asp Asn Asn Ser His 50 55 60
15	Gly Thr His Val Ala Gly Thr Val Ala Ala Leu Asn Asn Ser Ile Gly 65 70 75 80
	Val Leu Gly Val Ala Pro Ser Ala Ser Leu Tyr Ala Val Lys Val Leu 85 90 95
20	Gly Ala Asp Gly Ser Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu 100 105 110
	Trp Ala Ile Ala Asn Asn Met Asp Val Ile Asn Met Ser Leu Gly Gly 115 120 125
25	Pro Ser Gly Ser Ala Ala Leu Lys Ala Ala Val Asp Lys Ala Val Ala 130 135 140
30	Ser Gly Val Val Val Val Ala Ala Ala Gly Asn Glu Gly Thr Ser Gly 145 150 155 160
	Ser Ser Ser Thr Val Gly Tyr Pro Gly Lys Tyr Pro Ser Val Ile Ala 165 170 175
35	Val Gly Ala Val Asp Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Val 180 185 190
	Gly Pro Gln Leu Asp Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr 195 200 205
40	Leu Pro Gly Asn Lys Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Ser 210 215 220
45	Pro His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Asn 225 230 235 240
	Trp Thr Asn Thr Gln Val Arg Ser Ser Leu Gln Asn Thr Thr Thr Lys 245 250 255
50	Leu Gly Asp Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala 260 265 270
55	Ala Ala Gln 275

What is claimed is

1. A subtilisin 309 variant having a modified amino acid sequence of subtilisin 309 wild-type amino acid sequence, characterized in that the modified amino acid sequence comprises a substitution at one or more of positions 193, 194, 195, 196, 197, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 or 214, wherein
  - a. when a substitution occurs at position 193, the substituting amino acid is Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
  - b. when a substitution occurs at position 194, the substituting amino acid is His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
  - c. when a substitution occurs at position 195, the substituting amino acid is Gly, Gln, Asn, Ser, Asp or Glu;
  - d. when a substitution occurs at position 196, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu;
  - e. when a substitution occurs at position 197, the substituting amino acid is Met, Cys, Ala, His, Pro, Gly, Gln, Ser, Asp or Glu;
  - f. when a substitution occurs at position 199, the substituting amino acid is Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
  - g. when a substitution occurs at position 200, the substituting amino acid is 200 Asn or Ser;
  - h. when a substitution occurs at position 201, the substituting amino acid is Asp or Glu;
  - i. when a substitution occurs at position 202, the substituting amino acid is Pro, Gly, Gln, Asn, Ser, Asp or Glu;
  - j. when a substitution occurs at position 203, the substituting amino acid is Ile, Met, Ala, His, Pro, Gln, Asn, Ser, or Asp;
  - k. when a substitution occurs at position 204, the substituting amino acid is Gly, Gln, Asn, Ser, Asp or Glu;
  - l. when a substitution occurs at position 205, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu;
  - m. when a substitution occurs at position 206, the substituting amino acid is Asp or Glu;
  - n. when a substitution occurs at position 207, the substituting amino acid is Pro, Gly, Gln, Asn, Ser or Glu;
  - o. when a substitution occurs at position 208, the substituting amino acid is Leu, Ile, Val, Met, Cys, Ala, His, Pro, Gly, Gln, Asn, Asp or Glu;

- p. when a substitution occurs at position 209, the substituting amino acid is His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
- q. when a substitution occurs at position 210, the substituting amino acid is Asp or Glu;
- r. when a substitution occurs at position 211, the substituting amino acid is Ile, Val, Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, or Asp;
- s. when a substitution occurs at position 212, the substituting amino acid is Glu;
- t. when a substitution occurs at position 213, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu; and
- u. when a substitution occurs at position 214, the substituting amino acid is Pro, Gly, Gln, Asn, Asp, Ser or Glu;

whereby the subtilisin 309 variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to wild-type subtilisin 309; preferably

- i. when a substitution occurs at position 200, the substituting amino acid is Ser;
- ii. when a substitution occurs at position 205, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu;
- iii. when a substitution occurs at position 208, the substituting amino acid is Leu, Ile, Val, Met, Cys, Ala, His, Pro, Gly, Gln, Asn, Asp or Glu;
- iv. when a substitution occurs at position 209, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu; and
- v. when a substitution occurs at position 210, the substituting amino acid is Asp or Glu;

more preferably when a substitution occurs at one or more of positions 193, 194, 195, 196, 197, 199, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, or 214, the substituting amino acid for any of positions 193, 194, 195, 196, 197, 199, 201, 202, 204, 205, 206, 208, 209, 210, 211, 212, 213, or 214 is Asp or Glu; when a substitution occurs at position 203, the substituting amino acid for position 203 is Asp; and when a substitution occurs at one or more of positions 207 or 212, the substituting amino acid for either of positions 207 or 212 is Glu.

2. The subtilisin 309 variant of Claim 1 having a single amino acid substitution wherein the substitution is:

- a. Glu for Thr at position 207,
- b. Glu for Ser at position 210, or
- c. Asp for Ser at position 210.

3. A subtilisin 309 variant having a modified amino acid sequence of subtilisin 309 wild-type amino acid sequence, characterized in that the modified amino acid sequence comprises a substitution at three or more of positions 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 or 214 is substituted, wherein

- a. when a substitution occurs at position 193, the substituting amino acid is Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
- b. when a substitution occurs at position 194, the substituting amino acid is His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
- c. when a substitution occurs at position 195, the substituting amino acid is Gly, Gln, Asn, Ser, Asp or Glu;
- d. when a substitution occurs at position 196, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu;
- e. when a substitution occurs at position 197, the substituting amino acid is Met, Cys, Ala, His, Pro, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
- f. when a substitution occurs at position 198, the substituting amino acid is Glu, Gln, Asp or Ser;
- g. when a substitution occurs at position 199, the substituting amino acid is Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
- h. when a substitution occurs at position 200, the substituting amino acid is Asn, Ser Glu, or Asp;
- i. when a substitution occurs at position 201, the substituting amino acid is Asp or Glu;
- j. when a substitution occurs at position 202, the substituting amino acid is Pro, Gly, Gln, Asn, Ser, Asp or Glu;
- k. when a substitution occurs at position 203, the substituting amino acid is Leu, Ile, Val, Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
- l. when a substitution occurs at position 204, the substituting amino acid is Gly, Gln, Asn, Ser, Asp or Glu;
- m. when a substitution occurs at position 205, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu;

- n. when a substitution occurs at position 206, the substituting amino acid is Asp or Glu;
- o. when a substitution occurs at position 207, the substituting amino acid is Pro, Gly, Gln, Asn, Ser, Asp or Glu;
- p. when a substitution occurs at position 208, the substituting amino acid is Leu, Ile, Val, Met, Cys, Thr, Ala, His, Pro, Gly, Gln, Asn, Asp or Glu;
- q. when a substitution occurs at position 209, the substituting amino acid is His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
- r. when a substitution occurs at position 210, the substituting amino acid is Asp or Glu;
- s. when a substitution occurs at position 211, the substituting amino acid is Ile, Val, Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Glu, or Asp;
- t. when a substitution occurs at position 212, the substituting amino acid is Gln, Ser, Asp, or Glu;
- u. when a substitution occurs at position 213, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu; and
- v. when a substitution occurs at position 214, the substituting amino acid is Pro, Gly, Gln, Asn, Asp, Ser or Glu;

whereby the 309 variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to wild-type subtilisin 309; preferably

- i. when a substitution occurs at position 200, the substituting amino acid is Asn, Glu, Asp, or Ser;
- ii. when a substitution occurs at position 204, the substituting amino acid is Gly, Gln, Asn, Ser, Asp or Glu;
- iii. when a substitution occurs at position 205, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu;
- iv. when a substitution occurs at position 208, the substituting amino acid is Leu, Ile, Val, Met, Cys, Ala, His, Pro, Gly, Gln, Asn, Asp or Glu;
- v. when a substitution occurs at position 209, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu; and
- vi. when a substitution occurs at position 210, the substituting amino acid is Glu or Asp .



4. The subtilisin 309 variant of Claim 3 comprising two amino acid substitutions, wherein the substitutions are:

- a. Glu for Thr at position 207 and Glu for Ser at position 210;
- b. Leu for Val at position 197 and Glu for Ser at position 210; or
- c. Leu for Val at position 199 and Asp for Ser at position 210.
- d. Ala for Pro at position 204 and Thr for Ala at position 209;
- e. Phe for Tyr at position 208 and Asn for Leu at position 211;
- f. Asp for Asn at position 74 and Glu for Ser at position 210; or
- g. Glu for Lys at position 207 and Glu for Ala at position 210

5. A subtilisin 309 variant having a modified amino acid sequence of subtilisin 309 wild-type amino acid sequence, characterized in that the modified amino acid sequence comprises a substitution at three or more of positions 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 or 214 is substituted, wherein

- a. when a substitution occurs at position 193, the substituting amino acid is Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
- b. when a substitution occurs at position 194, the substituting amino acid is His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
- c. when a substitution occurs at position 195, the substituting amino acid is Gly, Gln, Asn, Ser, Asp or Glu;
- d. when a substitution occurs at position 196, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu;
- e. when a substitution occurs at position 197, the substituting amino acid is Met, Cys, Ala, His, Pro, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
- f. when a substitution occurs at position 198, the substituting amino acid is Glu, Gln, Asp or Ser;
- g. when a substitution occurs at position 199, the substituting amino acid is Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
- h. when a substitution occurs at position 200, the substituting amino acid is Asn, Ser Glu, or Asp;
- i. when a substitution occurs at position 201, the substituting amino acid is Asp or Glu;
- j. when a substitution occurs at position 202, the substituting amino acid is Pro, Gly, Gln, Asn, Ser, Asp or Glu;

- k. when a substitution occurs at position 203, the substituting amino acid is Leu, Ile, Val, Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
- l. when a substitution occurs at position 204, the substituting amino acid is Gly, Gln, Asn, Ser, Asp or Glu;
- m. when a substitution occurs at position 205, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu;
- n. when a substitution occurs at position 206, the substituting amino acid is Asp or Glu;
- o. when a substitution occurs at position 207, the substituting amino acid is Pro, Gly, Gln, Asn, Ser, Asp or Glu;
- p. when a substitution occurs at position 208, the substituting amino acid is Leu, Ile, Val, Met, Cys, Thr, Ala, His, Pro, Gly, Gln, Asn, Asp or Glu;
- q. when a substitution occurs at position 209, the substituting amino acid is His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;
- r. when a substitution occurs at position 210, the substituting amino acid is Asp or Glu;
- s. when a substitution occurs at position 211, the substituting amino acid is Ile, Val, Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Glu, or Asp;
- t. when a substitution occurs at position 212, the substituting amino acid is Gln, Ser, Asp, or Glu;
- u. when a substitution occurs at position 213, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu; and
- v. when a substitution occurs at position 214, the substituting amino acid is Pro, Gly, Gln, Asn, Asp, Ser or Glu;

whereby the 309 variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to wild-type subtilisin 309; preferably

- i. when a substitution occurs at position 200, the substituting amino acid is Asn, Glu, Asp, or Ser;
- ii. when a substitution occurs at position 204, the substituting amino acid is Gly, Gln, Asn, Ser, Asp or Glu;
- iii. when a substitution occurs at position 205, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu;

- iv. when a substitution occurs at position 208, the substituting amino acid is Leu, Ile, Val, Met, Cys, Ala, His, Pro, Gly, Gln, Asn, Asp or Glu;
- v. when a substitution occurs at position 209, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu; and
- vi. when a substitution occurs at position 210, the substituting amino acid is Glu or Asp.

6. The subtilisin 309 variant of Claim 5 comprising three amino acid substitutions, wherein the substitutions are:

- a. Glu substituted for Gln at position 200, Ala substituted for Gly at position 205, and Glu substituted for Ser at position 210;
- b. Leu substituted for Val at position 199, Ala substituted for Pro at position 204, and Glu substituted for Thr at position 207;
- c. Asp for Asn at position 74, Glu substituted for Gln at position 200, and Glu substituted for Ser at position 210; or
- d. Glu for Gln at position 200, Glu for Thr at position 207, and Glu for Ser at position 210.

7. The subtilisin 309 variant of Claim 5 having the following four substitutions: Ala substituted for Pro at position 204, Glu substituted for Thr at position 207, Glu substituted for Ser at position 210, and Asp substituted for Asn at position 74.

8. The subtilisin 309 variant of Claim 5 having the following five substitutions: Leu for Ile at position 199, Ala for Pro at position 204, Glu for Lys at position 207, Glu for Ala at positions 210, and Asp substituted for Asn at position 74.

9. A subtilisin 309 variant having a modified amino acid sequence of subtilisin 309 wild-type amino acid sequence, the wild-type amino acid sequence comprising a first loop region, a second loop region, a third loop region, a fourth loop region and a fifth loop region; characterized in that the modified amino acid sequence comprises a substitution at one or more positions in one or more of the loop regions; wherein

- A. when a substitution occurs in the first loop region, the substitution occurs at one or more of positions 57, 58, 59, 60, 61, 63, or 64; wherein

- a. when a substitution occurs at position 57, the substituting amino acid is Asn, Asp, Glu or Ser;
  - b. when a substitution occurs at position 58, the substituting amino acid is Glu;
  - c. when a substitution occurs at position 59, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
  - d. when a substitution occurs at position 60, the substituting amino acid is Asp, Gln, Glu or Ser;
  - e. when a substitution occurs at position 61, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
  - f. when a substitution occurs at position 63, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser; and
  - g. when a substitution occurs at position 64, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser;
- B. when a substitution occurs in the second loop region, the substitution occurs at one or more of positions 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104 or 105; wherein
- a. when a substitution occurs at position 93, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr;
  - b. when a substitution occurs at position 94, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser, Thr or Val;
  - c. when a substitution occurs at position 95, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
  - d. when a substitution occurs at position 96, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr;
  - e. when a substitution occurs at position 97, the substituting amino acid is Asp or Glu;
  - f. when a substitution occurs at position 98, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
  - g. when a substitution occurs at position 99, the substituting amino acid is Asp or Glu;
  - h. when a substitution occurs at position 100, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
  - i. when a substitution occurs at position 101, the substituting amino acid is Asp or Glu;

- j. when a substitution occurs at position 102, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr;
  - k. when a substitution occurs at position 103, the substituting amino acid is Asp or Glu;
  - l. when a substitution occurs at position 104, the substituting amino acid is Asp or Glu;
  - m. when a substitution occurs at position 105, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Leu, Met, Pro, Ser, Thr or Val; and
- C. when a substitution occurs in the third loop region, the substitution occurs at one or more of positions 124, 125, 126, 127, 128, 129, 130 or 131; wherein
- a. when a substitution occurs at position 124, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser, Thr or Val;
  - b. when a substitution occurs at position 125, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
  - c. when a substitution occurs at position 126, the substituting amino acid is Asp or Glu;
  - d. when a substitution occurs at position 127, the substituting amino acid is Asn, Asp, Gln, Glu, Gly or Ser;
  - e. when a substitution occurs at position 128, the substituting amino acid is Asp or Glu;
  - f. when a substitution occurs at position 129, the substituting amino acid is Asn, Asp, Gln, Glu, Gly or Ser;
  - g. when a substitution occurs at position 130, the substituting amino acid is Asp or Glu; and
  - h. when a substitution occurs at position 131, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser, Thr;
- D. when a substitution occurs in the fourth loop region, the substitution occurs at one or more of positions 152, 153, 154, 155, 156, 157, 158, 159, 160 or 161; wherein
- a. when a substitution occurs at position 152, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
  - b. when a substitution occurs at position 153, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

- c. when a substitution occurs at position 154, the substituting amino acid is Asp or Glu;
  - d. when a substitution occurs at position 155, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
  - e. when a substitution occurs at position 156, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr;
  - f. when a substitution occurs at position 157, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
  - g. when a substitution occurs at position 158, the substituting amino acid is Asp or Glu;
  - h. when a substitution occurs at position 159, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Leu, Met, Pro, Ser, Thr or Val;
  - i. when a substitution occurs at position 160, the substituting amino acid is Asp or Glu; and
  - j. when a substitution occurs at position 161, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr or Val;
- E. when a substitution occurs in the fifth loop region, the substitution occurs at one or more of positions 181, 182, 183, 184 or 185; wherein
- a. when a substitution occurs at position 181, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr;
  - b. when a substitution occurs at position 182, the substituting amino acid is Asp or Glu;
  - c. when a substitution occurs at position 183, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr, Tyr or Val;
  - d. when a substitution occurs at position 184, the substituting amino acid is Asp or Glu; and
  - e. when a substitution occurs at position 185, the substituting amino acid is Asn, Asp, Glu or Ser;

whereby the subtilisin 309 variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to wild-type subtilisin 309.

10. The subtilisin 309 variant of Claim 9, wherein the subtilisin 309 wild-type amino acid sequence further comprises a sixth loop region, wherein the modified amino acid sequence further comprises one or more substitutions

in the sixth loop region; wherein the substitution(s) in the sixth loop region occurs at one or more of positions 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 or 214; wherein

- a. when a substitution occurs at position 193, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr;
- b. when a substitution occurs at position 194, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr;
- c. when a substitution occurs at position 195, the substituting amino acid is Asn, Asp, Gln, Glu, Gly or Ser;
- d. when a substitution occurs at position 196, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
- e. when a substitution occurs at position 197, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr;
- f. when a substitution occurs at position 198, the substituting amino acid is Asp, Gln, Glu or Ser;
- g. when a substitution occurs at position 199, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr;
- h. when a substitution occurs at position 200, the substituting amino acid is Asn, Asp, Glu or Ser;
- i. when a substitution occurs at position 201, the substituting amino acid is Asp or Glu;
- j. when a substitution occurs at position 202, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser;
- k. when a substitution occurs at position 203, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr or Val;
- l. when a substitution occurs at position 204, the substituting amino acid is Asn, Asp, Gln, Glu, Gly or Ser;
- m. when a substitution occurs at position 205, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
- n. when a substitution occurs at position 206, the substituting amino acid is Asp or Glu;
- o. when a substitution occurs at position 207, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser;

- p. when a substitution occurs at position 208, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr or Val;
- q. when a substitution occurs at position 209, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr;
- r. when a substitution occurs at position 210, the substituting amino acid is Asp or Glu;
- s. when a substitution occurs at position 211, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser, Thr or Val;
- t. when a substitution occurs at position 212, the substituting amino acid is Asp, Gln, Glu or Ser;
- u. when a substitution occurs at position 213, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser; and
- v. when a substitution occurs at position 214, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

11. A cleaning composition selected from the group consisting of a hard surface cleaning composition, a dishwashing composition, an oral cleaning composition, a denture cleansing composition, a contact lens cleaning composition and a fabric cleaning composition, characterized in that the cleaning composition comprises the subtilisin 309 variant of any of Claims 1-11 and a cleaning composition carrier.

12. The subtilisin 309 variant of any of Claims 1-11 wherein the modified amino acid sequence further comprises His or Asp substituted for Asn at position 74.

13. The cleaning composition of Claim 11, wherein the cleaning composition is a hard surface cleaning composition.

14. The cleaning composition of Claim 11; wherein the cleaning composition is a fabric cleaning composition; preferably the composition is in the form of a liquid.

15. A DNA sequence 309 gene encoding the subtilisin 309 variant of any of Claims 1-11.



1/2

## Comparison of subtilisin sequences from:

*B.amyloliquefaciens**B.lentus*

01	10	20	30
A Q S V P Y G V S Q I K A P A L H S Q C Y T C S N V K V A V I D S G I D S S H P			
A Q S V P W G I S R V Q A P A A H N R G L T C S G V K V A V L D T G I S T * H P			
41	50	60	70
D L K V A C C A S M V P S E T N P F Q D N N S H G T H V A G T V A A L N N S I G			
D L N I R G G A S F V P G E * P S T Q D G N G H G T H V A G T I A A L N N S I G			
81	90	100	110
V L G V A P S A S L Y A V K V L G A D G S C Q Y S W I I N G I E W A I A N N H D			
V L G V A P S A E L Y A V K V L G A S G S C S V S S I A Q C L E W A G N N G H H			
121	130	140	150
V I N M S L G G P S G S A A L K A A V D K A V A S G V V V A A A G N E G T S G			
V A N L S L G S P S P S A T L E Q A V N S A T S R G V L V A A S G N S G A G S			

FIG 1/A

161 170 180 190  
SSSTVGYPGKYPSVIAVGAVDSSNQRASSFSSVGPELDVMA  
\*\*\*ISYPARYANAMAVGATDQNNNRASFSQYGACLDIVA  
201 210 220 230  
PGVSIQSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPN  
PCVNVQSTYPGSTYASLNGTSMATPHVAGAAALVKQKNPS  
241 250 260 270  
WTNTQVRSSLENTTTKLGDSFYYGKGLINVQAAAO  
WSNVQIRNHLKNTATSLGSTNLVCSGLVNAEATR

FIG 1/B